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CRANIOFACIAL ANOMALIES IN CHILDREN:
Diagnosis, Management, Outcomes

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I acknowledge the support and teaching of Dr David Netherway the senior scientist at the Australian Craniofacial Unit, and Professor Grant Townsend at Adelaide Dental Hospital, who together developed my interest in morphological measurement using 3D CT scans and who helped me translate this technical expertise to answer clinical questions.

I acknowledge the permissions given in respect to copyright law given by the Journal of Craniofacial Surgery, the Cleft Plate Craniofacial Journal and the other publishers to allow the incorporation of the papers published in their journals into this thesis.

I should like to thank my students from Adelaide, Brisbane, Sydney, Kota Bharu (Universiti Sains Malaysia), and Shanghai. It has been a privilege to know all of them, whether they be from a medical, dental or

a scientific background. Their different academic and cultural training has enriched my experience of the perception of facial anomalies and their searching questions continually prompts my search for answers.

I should like to thank my patients and their families in Australia, the United Kingdom, China, Indonesia, Vietnam and Malaysia who are a constant reminder for the need to improve our understanding of disease processes involved in Craniofacial anomalies so that we might improve their management and outcomes.

Finally, I should like to thank Mandy, Hazel, Emily and Ross for their continued support and forbearance with me and the resultant domestic upheaval, without their tolerance this work could not have been undertaken.

DECLARATION

"I declare that the contents of this thesis, submitted to the University of Edinburgh for the degree of Doctor of Science, were composed entirely by myself except where otherwise stated in Appendix One. I confirm that none of the material presented in these papers forms part any other degree (specifically either the M.D. thesis or the Ph.D. thesis submissions).

Peter John Anderson

DEDICATION

This thesis is dedicated to the memory of Azuan Mohd Alias (“ Harry”)
who had Proteus syndrome.

He was the most remarkable and courageous young man who before his
untimely death displayed incredible tolerance and forbearance of my
inability to adequately understand his disease process and to provide
only limited management, which resulted ultimately in an unsuccessful
outcome.

His memory is a reminder to to me to constantly strive to increase the
knowledge and understanding of Craniofacial disease processes with a
view to providing targeted treatment leading to improved outcomes for
children with craniofacial anomalies.

ABSTRACT

The causes of facial anomalies in children may be congenital, traumatic, oncologic (or in some cases) remain unknown. Many of these craniofacial conditions warrant further elucidation of the clinical features, to allow accurate diagnosis and targeted management. Long term outcome studies of children with craniofacial anomalies, are essential to evaluate treatment protocols and to aid those who treat affected children to improve and advance their standards of care. These objectives require clinicians, along with their scientific colleagues to strive to increase their recognition of morphological anomalies and understanding of the underlying disease processes, so as to develop specific management strategies. The aim is to find new answers to improve the quality of life of affected children throughout the world. This collection of papers has been prompted by a desire to contribute towards that goal.

INTRODUCTION

The development of the new surgical discipline of Craniofacial Surgery came about due to failure of existing surgical specialties to adequately address complex deformities of the cranium and the facial skeleton. The established disciplines of Neurosurgery, Plastic surgery and Oral and Maxillofacial surgery could deal with some aspects of the management but not all. Paul Tessier in conjunction with his Neurosurgical colleague Guillot were the first to attempt to resolve the surgical challenge of the cranial-orbital complex using their combined skills. After receiving world attention the goals and practice of Craniofacial surgery were set down by those surgeons with a major interest in the discipline at the inauguration of International Society of Craniofacial Surgeons in 1982.

The specialty of Craniofacial surgery is still young, its founding father Paul Tessier only recently passed way, and his disciples, the first generation of surgeons who were the founders of the International society of Craniofacial surgeons have just retired. Much of the work highlighted by Paul Tessier as encompassing this specialty has (and indeed still is in some centres) been undertaken by longer established surgical specialties of Neurosurgery, Plastic surgery, Oral and Maxillofacial surgery, so the discipline of Craniofacial surgery could be considered to be a hybrid of these disciplines.

This thesis is based on the continuing search to improve the understanding of the many processes which result in deformity of the craniofacial region with the aim to improve management.

CHAPTER ONE

INTRODUCTION

Craniosynostosis can affect all human populations and its management is the fundamental disease process treated by craniofacial surgeons. The disease process where the cranial sutures fuse prematurely, has long been recognised¹. The early attempt to explain the resultant cranial morphologies² have impacted on treatment philosophy, but the results of treatment were inconsistent. Recently, there has been the recognition of the importance of genetic mutations associated with the disease in the commonest syndromic conditions³, but despite these discoveries the intracellular mechanisms which result in premature suture fusion for most children still require elucidation.

The treatment of fusion by surgical removal of the affected sutures was first undertaken in 1892⁴. Initially, the surgery remained in the domain of the Neurosurgeons, but the realisation that improved outcomes could be obtained by addressing the secondary morphological changes in the calvarium and skull base management led to the involvement of dedicated craniofacial surgeons, who together with their neurosurgical colleagues now provide a comprehensive management service, in dedicated units.

This collection of papers extend the knowledge of the clinical features of children with craniosynostosis, identifies management challenges and investigates long term outcomes.

1. Otto AW (1830) Lehrbuch der pathologischen Anatomie.

Rucker, Berlin.

2. Virchow R (1851) Ueber den Cretinismus, namenlich in Franken, und ueber pathologische Scadelformen.

Verh Phys Med Ges Wutzburg **2**: 230 – 271.

3. Reardon W, Winter RM, Rutland P, Pulleyn LJ, Jones B, Malcolm S (1995) Mutations in the fibroblastic growth factor receptor type 2 gene cause Crouzon syndrome.

Nature Genetics **9**: 98 – 103.

4. Lane LC (1892) Pioneer craniectomy for the relief of mental imbecility due to premature sutural closure and microcephalus.

Journal of the American Medical Association **18**: 49 - 50.

CHAPTER ONE - PAPERS

The first paper is a report of two cases from different ethnic groups of a new previously unidentified craniosynostosis syndrome. The number of craniosynostosis syndromes continues to increase with over 300 recorded on the London dysmorphology database. Better recognition of these specific phenotypes will aid treatment planning and management.

The second paper is a review of the clinical features of a series of patients with a rare craniosynostosis condition, Antley-Bixler syndrome. Because of the rarity the collaborative study was undertaken by the first author using the resources of two of the world's major Craniofacial Units, Great Ormond Street Hospital, London and Australian Craniofacial Uni, Adelaide. This study is the first reported series of patients, rather than isolated case reports, and highlights the complex and differing underlying genetic anomalies which can result in similar phenotypes.

The third paper is a case report which reports anomalous venous drainage which can result in association with non-syndromic craniosynostosis. It emphasizes the risks of surgical management in such cases. It also underlines the complex relationship between craniosynostosis, altered calvarial morphology and the intracranial vascular anatomy and brain function. These complex relationships still require elucidation.

The fourth paper is a collaborative study to investigate the Ophthalmological findings in Apert syndrome patients prior to any surgical intervention. Its findings reflect the natural history and consequences if surgical intervention is delayed. This review is important in that it aids the decision not just how to intervene, but, critically provides a guide as to the timing of such interventions.

The fifth paper is a study to investigate phenotypic differences in the Ophthalmological aspects of Apert syndrome. Currently, it is known that 99% of Apert syndrome cases have one of two underlying mutations in the *FGFR2* gene. There still remains controversy as to whether there are subtle phenotypic differences between the two groups. This study extends this investigation into a specific area compares the Ophthalmological findings in the two groups. It was the first study of its type and did not identify any clear clinical differences between the two genotypes.

The sixth paper is a clinical research study reviewing the post temperature course in patients who have undergone transcranial surgery for non-syndromic craniosynostosis. This study resulted from the common clinical observation that many children develop an apparent pyrexia post-operatively, yet despite extensive investigations no evidence of local or systemic infection can be found. It was also recognised that in other surgical disciplines, a similar phenomenon had been identified. This study found that this was common in all forms of

non-syndromic craniosynostosis and regardless of gender. The results have led to an alteration in clinical practice with much higher threshold before invasive or radiological investigations are undertaken in the early post-operative phase of a child's recovery.

The seventh paper builds on the previous study extending the scope of the investigation to syndromic craniosynostosis. Again careful study of post-operative temperature changes following transcranial surgery in patients in this study with Pfeiffer syndrome. The findings confirmed a postoperative "double peak" which appeared to be part of the normal uncomplicated recovery course.

The eighth paper extends this study further to review the post operative temperature changes in Apert syndrome patients. These patients are potentially more complex with the underlying propensity for anatomical anomalies of the central nervous system co-existing as part of the underlying condition. Not surprisingly then this study identified a more complex post-operative course than in the previous papers, and significantly it identified that a third post-operative peak in temperature which was a good indicator that there could be an underlying infective process, something which remains very difficult to detect clinically in Apert syndrome children.

The ninth paper reviews the management of secondary craniosynostosis occurring as a result of ventricular shunting by neurosurgeons in infancy and was undertaken in conjunction with my

neurosurgical colleagues. As a phenomenon this is often overlooked by clinicians, partly because it is uncommon but also because it could be considered an iatrogenic injury. There have been few studies undertaken to date into its management and this paper reviews the Adelaide Craniofacial Unit's experience, and proposes guidelines for deciding whether to undertake calvarial reconstruction in such cases.

The tenth paper is modification of surgical technique for simultaneous midface advancement in children with midface hypoplasia and brachycephaly resulting from syndromic craniosynostosis. It reports when completing a Le fort III osteotomy as part of a frontofacial advancement applying multiple distractors to optimally correct both the supraorbital region and the midface, rather than compromise between these two surgical goals, which is necessary in conventional monobloc frontofacial surgery.

The eleventh paper is collaborative outcomes paper studying the Ophthalmological results of protocol management of Crouzon patients. This review highlights the importance of the timing of orbital surgical interventions to produce a good outcomes.

The twelfth paper is a study similar to paper eleven only this time it focuses on longterm visual outcomes in Apert syndrome patients. It demonstrates that surgical interventions around the orbits improve the natural history, but again timing is important for good outcomes.

The thirteenth paper is a collaborative outcome study with the University of Adelaide department of Psychology, investigating Crouzon, Pfeiffer and Muencke syndrome patients with psychological assessments at skeletal maturity. It focuses on intellectual and social outcomes rather than the aesthetic outcomes which Surgeons traditionally use as an outcome measure. This paper demonstrates that patients can live and work independently and while most complete secondary education some can achieve academic success at tertiary level.

The fourteenth paper is again a longterm outcomes study reviewing the results of a cohort of patients with unicoronal synostosis treated using the same surgical protocol who have all reached skeletal maturity. This identified that most cases had a an acceptable aesthetic outcome although a few required re-operation for recurrent raised intracranial pressure. However, revisional surgery, particularly to help correct strabismus, was much higher than had been expected. This paper underlines the importance of longterm multidisciplinary follow up by craniofacial surgeons team in conjunction with the ophthalmology team.

The fifteenth and final paper in this chapter is a multicentre-collaborative study looking at the very long outcome for children with Saethre-Chotzen syndrome. It was reported in European literature that the underlying mutation in the *TWIST* gene predisposed affected

females to an increased risk of breast cancer in adulthood, something which anecdotally didn't appear valid.

Collaborating with colleagues in Clinical Genetics throughout Australia an investigative study into mutation positive adult females was undertaken and found no evidence of increased cancer risk. While this study affects adults it is important for managing children since they and their parents may need reassurance if they have heard of the association. Also if this study had confirmed an association, then management would have had to include counselling and possible referral to breast surgeons to discuss possible prophylactic mastectomies.

In summary this collection of papers has contributed to the understanding of the clinical presentation of craniosynostosis, has widened and refined the possible management options and study of the longterm outcomes modify and enhance existing treatment protocols.

A New Syndrome With Craniosynostosis and Cleft Lip and Palate

Peter J. Anderson, MD, PhD, FDSRCS(Ed), FRCS(Plast), FRACS,*† Eric A. Haan, FRCP,†‡ and David J. David, MD, FRCS, FRACS*

Abstract: Two unrelated girls with craniosynostosis and bilateral cleft lip and palate who also had developmental delay and umbilical herniae are presented. We propose that these patients have the same condition, and that their combination of features may constitute a new syndrome. Management of the patients is discussed.

Key Words: Craniosynostosis, cleft lip and palate, syndrome

(*J Craniofac Surg* 2011;22: 122–124)

PATIENT 1

A 5-month-old female infant, the first child of unrelated parents, was referred to the Australian Craniofacial Unit (ACFU) for assessment and treatment. She was born at 38 weeks by cesarean delivery for breech presentation, with a birth weight of 3920 g (75th–90th centile). The pregnancy was otherwise unremarkable, and the only notable family history was that the father had an aunt with unilateral cleft lip and palate.

Craniofacial features described at birth were bilateral cleft lip and palate and brachycephaly. There were feeding difficulties, and she remained in her local hospital for a month until feeding was established. At 2 months of age, she underwent a limited bilateral coronal suture excision by a local neurosurgeon, and at 3 months, bilateral inguinal herniae were repaired.

After transfer to the ACFU in Adelaide at 4 months of age, she underwent a multidisciplinary assessment including craniofacial, neurosurgical, pediatric, cardiac ophthalmologic, otolaryngologic, and clinical genetic assessments. Weight was below the third centile, and length was on the 75th centile. She had bicoronal synostosis with metopic bulging, bilateral external auditory canal stenosis, prominent ear crura, small ear lobules, flattened supraorbital margins, mild proptosis, hypotelorism, down-slanting and short palpebral fissures, epicanthic folds, and bilateral cleft lip and palate with severe midface hypoplasia (Fig. 1). The anomalies outside the craniofacial region were less marked and included an umbilical hernia and mild soft tissue syndactyly of the second web space on her hands (Fig. 2). Her development was delayed.

Radiologic examination of the skull identified an oxycephaly with hydrocephalus and metallic clips in situ from the previous neurosurgical interventions in the coronal sutures. The lambdoid sutures were noted to be fusing. Radiologic examination of the cervical spine, the chest, and the limbs was unremarkable. Her clinical features did not fit with a recognized syndrome. Karyotype was normal.

She subsequently underwent staged surgical repair beginning with transcranial modified fronto-orbital advancement with excision of the remaining fused sutures and calvarial remodeling. Her recovery was unremarkable, and 3 months later, at age 9 months, she underwent the second stage with repair of her bilateral cleft lip. She was discharged home (interstate) with arrangements for follow-up locally by the neurosurgical team and also by the pediatric surgeon for her umbilical hernia.

She was well until 4 months later when she was admitted to a local hospital to have a shunt placed. She developed *Streptococcus pneumoniae* meningitis and died 7 days later of her infection. There was no postmortem examination.

PATIENT 2

A 17-month-old Indonesian girl was referred to the ACFU for the management of craniosynostosis and bilateral cleft lip and palate. She was the third child of nonconsanguineous Indonesian parents, born after an uncomplicated pregnancy. Her 2 older sisters aged 5 and 9 years were both well. As a result of her obvious deformities, he had ongoing difficulties with feeding.

After transfer to ACFU, he underwent an extensive multidisciplinary assessment including craniofacial, neurosurgical, pediatric, cardiac ophthalmologic, otolaryngologic, and clinical genetic assessments. Her weight was 7050 g at 15 months (<third centile), length was 64 cm (<third centile), and head circumference was 39 cm (<second centile). The craniofacial anomalies included bicoronal synostosis

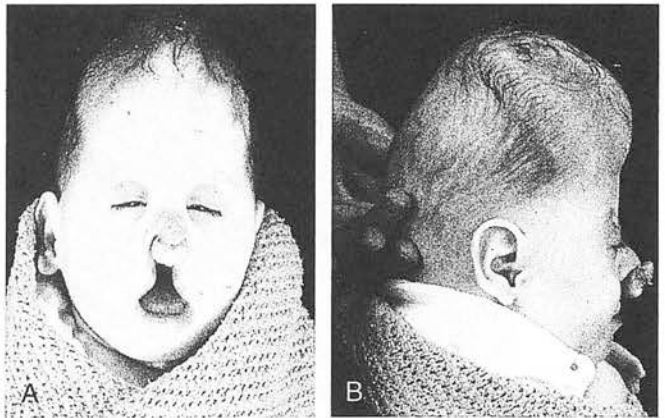


FIGURE 1. Patient 1 at presentation aged 5 months.

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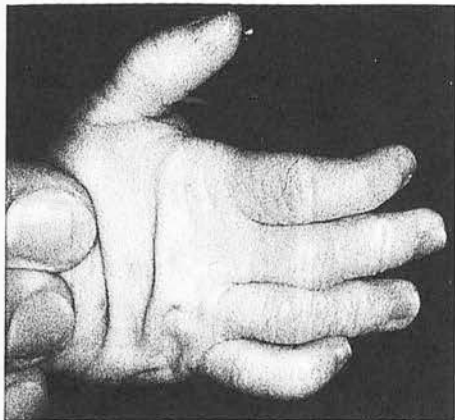


FIGURE 2. Patient 1 hand demonstrating syndactyly second web space.

with an absent vertex, flattened supraorbital margins, hypertelorism, short palpebral fissures, mild proptosis, and bilateral complete cleft lip and palate (Figs. 3 and 4). Other anomalies included limited upgaze, thought to be due to mechanical problems resulting from the distorted orbits, and an umbilical hernia (Fig. 5). Hands and feet were normal. There was a moderate developmental delay. A radiologic skeletal survey did not identify abnormalities other than those of the skull (Fig. 6). A magnetic resonance imaging study of the brain found a Chiari type 1 malformation, with a small foramen magnum. It also identified an anomalous course of the straight sinus.

The following investigations gave normal results: chromosome study; subtelomeric multiplex ligation dependent probe amplification analysis; screening by denaturing high-performance liquid chromatography for mutations in fibroblast growth factor receptor 1 (*FGFR1*) exon 7, *FGFR2* exons 7, 9, and 10, *FGFR3* exons 7 and 10, and *TWIST* exon 1; sequencing of *EFNB1* gene; and echocardiogram.

Management was aided by a nylon model of her skull (Fig. 7) and consisted of repair in 2 stages. First, decompression of the posterior fossa and the foramen magnum was undertaken by a neurosurgeon, with the patient positioned prone. During this procedure, it was noted that the cerebellar tonsils extended to C2 and were repositioned to the level of the foramen magnum. Postoperatively, it was noted that the left central retinal artery had become occluded during the procedure resulting in ischemia and blindness in that eye. Transcranial resection of the fused coronal and lambdoid sutures with calvarial remodeling was undertaken a week later. Postoperative recovery was unremarkable.

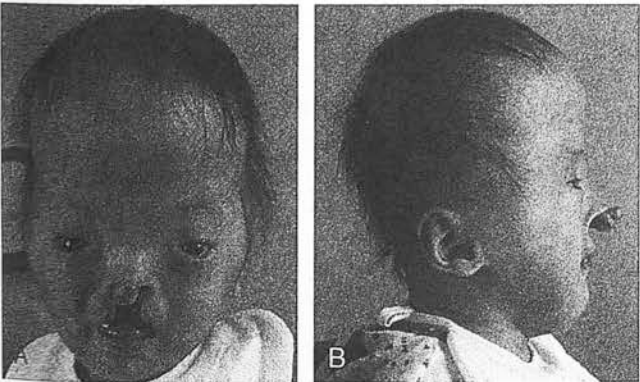


FIGURE 3. Patient 2 at presentation aged 17 months.



FIGURE 4. Patient 2 close-up view of the cleft lip/palate.

One month later, the cleft lip and palate were repaired simultaneously using techniques similar to those used to repair nonsyndromic bilateral cleft lip and palate. Postoperatively, her recovery was unremarkable and feeding improved, and she returned to Indonesia 6 weeks later. At outpatient follow-up 1 year later, she was thriving and continuing to develop. She had developed hypothyroidism that required medication but was otherwise well. She remains on regular outpatient review.

DISCUSSION

These 2 girls with craniosynostosis and bilateral cleft lip and palate, who also have developmental delay and herniae (umbilical in both and inguinal in one), may have a novel syndrome. One of the girls also had prenatal and postnatal growth failure, and one had a mild finger syndactyly. Inguinal and umbilical herniae are common in infants, and although we have included hernia as a feature of the proposed new syndrome, its relationship to the other features in these 2 patients remains uncertain.

The simultaneous presence of craniosynostosis and a cleft is recognized in Crouzon syndrome,¹ thanatophoric dysplasia,² and Saethre-Chotzen syndrome.³ Their clinical craniofacial features, absence of radiographic anomalies other than the skull in both patients and no identified mutation on *FGFR1-3* and *TWIST* in patient 2, suggest that their phenotypes do not reflect the variable expressivity of one of these clinical entities. A diagnosis of craniofrontonasal syndrome was considered for patient 2, but no mutation was identified in the *EFNB1* gene.⁴ The combination of lambdoid or coronal suture synostosis with cleft lip/palate has been reported in Michels syndrome,⁵ but the pattern of craniosynostosis and other features does not support this as a diagnosis. The combination of craniosynostosis and cleft lip and palate with limb shortening has been described in Herman syndrome,⁶ but again, this would seem to be a different

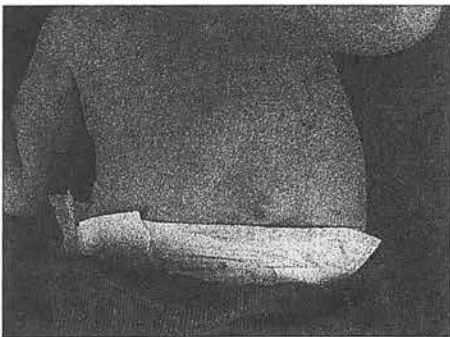


FIGURE 5. Patient 2 aged 17 months demonstrating the umbilical hernia.

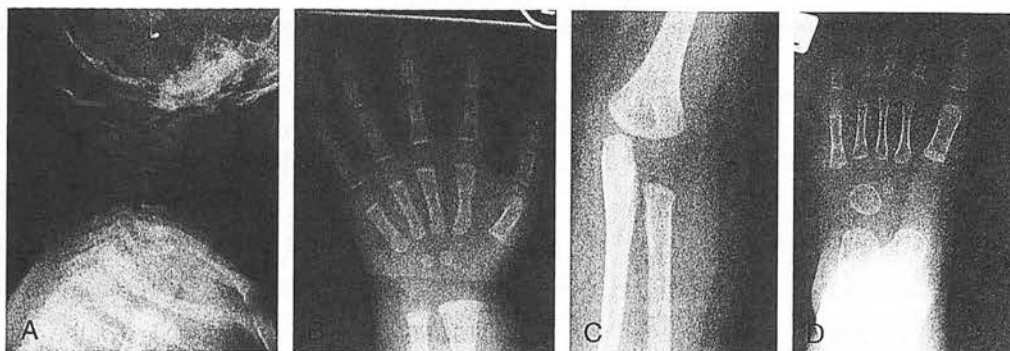


FIGURE 6. Skeletal survey of patient showing normal cervical spine, hand, elbow, and foot.

entity. The unusual pattern of failure of calvarial formation seems to be similar to that reported in craniomelic syndrome,⁷ but the patients we describe did not have limb shortening.

The craniofacial management strategy adopted in these patients was essentially similar, allowing for the difference in ages at presentation. The early excision of the affected sutures and cranial remodeling in infancy was followed later by surgical correction of the bilateral cleft lip and palate using the same conventional techniques used for nonsyndromic bilateral cleft lip and palate. Management of such complex cases can best be achieved in specialized

centers where coordinated multidisciplinary care can be delivered. In conclusion, we describe 2 patients with a distinct pattern of craniofacial abnormalities that may represent a previously unreported syndrome.

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The authors thank Prof Michael Buckley, South East Area Laboratory Services, Randwick, Sydney, Australia, for performing the *FGFR1-3* and *TWIST* testing; Prof Peter Wiecker of the Institute of Human Genetics, University of Magdeburg, Germany, for performing the *EFNB1* sequencing.

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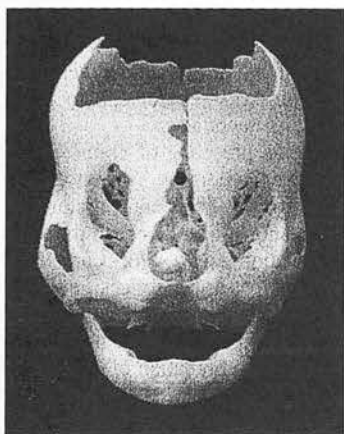


FIGURE 7. Patient 2 nylon model of the preoperative skull.

Spectrum of Antley-Bixler Syndrome

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David J. Dunaway, MB, ChB, BDS, FRCS, FDS RCS, FRCS(Plast),||
David J. David, AC, MD, FRCSE, FRCS, FRACS,*‡§ and
Peter J. Anderson, MD, PhD, FDS RCS, FRCS, FRCS(Plast), FRACS†‡§

Abstract: Antley-Bixler syndrome (ABS) is an exceptionally rare craniosynostosis syndrome characterized by radiohumeral synostosis present from the perinatal period. There is a wide spectrum of anomalies seen within ABS, and other features include midface hypoplasia; choanal stenosis or atresia; multiple joint contractures; visceral anomalies, particularly of the genitourinary system; and impaired steroidogenesis.

The condition of ABS is curious in that mutations of 2 separate genes have been identified and that there seem to be subtle phenotypic differences between the 2 genotypes. Mutations of the P450 oxidoreductase gene have been reported in those patients with genital anomalies and/or impaired steroidogenesis, and the S351C mutation of the fibroblast growth factor receptor 2 gene has been reported predominantly in those patients with normal genitalia and steroidogenesis. We report a series of 4 patients with ABS and review their main findings and management.

Key Words: Antley-Bixler syndrome, phenotype, POR, FGFR2

(*J Craniofac Surg* 2010;21: 1560–1564)

The aims of this study were to review a series of 4 new cases of Antley-Bixler syndrome (ABS) and to identify the range in phenotypic presentation in each case and their subsequent management. In particular, we studied the phenotypic differences between the 2 known genotypes.

In 1975, a patient with similar features to those seen in the acrocephalosyndactyly syndromes but who was sufficiently different so as to suggest a distinct entity was described by Ray Antley and David Bixler.¹ A case with similar features had previously been reported by Lacheretz et al,² and additional patients have since been described with what has come to be known as ABS. The 2 most consistent features are craniosynostosis and radiohumeral

synostosis present from the perinatal period, and these are generally considered to be the minimum diagnostic criteria.³

Other features that may be present include midface hypoplasia; choanal stenosis or atresia; multiple joint contractures; visceral anomalies, particularly of the genitourinary system; and impaired steroidogenesis.^{1,4–8} The spectrum of anomalies that has been reported to occur within ABS is wide as can be their severity.^{5,9}

Antley-Bixler syndrome is primarily a syndrome affecting the development of bone and cartilage, and it has been described as “a syndrome of multisynostotic osteodysgenesis.”¹⁰ A patient with ABS may have multisystem involvement including craniofacial, musculoskeletal, genitourinary, and, less commonly, cardiovascular and gastrointestinal anomalies, and consequently, optimal management of these patients requires a multidisciplinary team approach.^{6,8,11} Mortality has been reported to be as high as 80% within the neonatal period, primarily due to airway compromise, and prognosis improves with increasing age.^{11,12} Hence, those patients who survive infancy may demonstrate a less severe phenotype and consequently distort the spectrum of phenotypic severity in a series consisting of older individuals.

The condition of ABS is curious in that mutations within 2 separate genes have been identified and that there seem to be subtle phenotypic differences between the 2 genotypes. Mutations of the electron donor enzyme P450 oxidoreductase (POR) gene have been reported in those patients with the characteristic craniofacial manifestations and abnormal genitalia and/or impaired steroidogenesis, whereas the S351C mutation of the fibroblast growth factor 2 (FGFR2) gene has been reported predominantly in those patients with normal genitalia and steroidogenesis.^{3,13–16}

The S351C de novo heterozygous mutation of the FGFR2 gene was the first mutation to be reported in patients with ABS.³ It was subsequently noted that this mutation was not present in all patients examined and particularly in those with abnormal genitalia and/or impaired steroidogenesis.¹⁴ Genetic screening of those patients with ABS and abnormal genitalia and/or impaired steroidogenesis revealed several homozygous or compound heterozygous mutations of the POR gene including 2 missense mutations (R457H and Y578C), a 24–base pair deletion mutation (L612_W620delinsR) resulting in the removal of 9 amino acids and the insertion of 1 amino acid, a single–base pair insertion mutation (I444fsX449) resulting in a frameshift, and a silent mutation (G5G).^{13,15,16}

Four new cases of ABS surviving beyond the neonatal period from 2 separate craniofacial centers are reported, and their main clinical features and management are discussed including a phenotypic comparison of the 2 genotypes.

CLINICAL REPORTS

Patient 1

This female patient with karyotype 46XX was delivered at term by an elective cesarean section as the result of a pregnancy complicated by insulin-dependent diabetes mellitus and preeclampsia.

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Cranial morphology included a tall brachycephalic cranial vault with a degree of plagiocephaly, occipital flattening, and frontal bossing and a head circumference at birth at just the third percentile. There was midface hypoplasia, splayed nasal bones with a depressed nasal bridge, and a class III skeletal relationship. There was proptosis with an exotropia and bilateral lop ears with moderate conductive hearing loss. Bilateral choanal stenosis was present, and the child had a severe obstructive sleep apnea syndrome (OSAS). On formal neuropsychologic assessment in later childhood, there was a delay in expressive speech.

This child had bilateral radioulnar humeral synostosis, but there was good movement at the shoulders, wrists, and fingers. Hyperlordosis of the lumbar spine was present, and the gait was abnormal.

On examination at birth, the child was found to have ambiguous genitalia with fused labia and clitoromegaly, and a single urogenital sinus draining the bladder and vagina was found on further investigation.

Raised intracranial pressure (ICP) was demonstrated after insertion of an ICP monitor at 3 months of age. An Arnold-Chiari type I malformation was subsequently identified on magnetic resonance imaging. Echocardiogram revealed left ventricular hypertrophy with no outlet obstruction. Genetic screening revealed a single POR mutation.

Obstructive sleep apnea syndrome was not able to be relieved with continuous positive airway pressure; nasal stents were unsuccessful, and a tracheostomy was subsequently performed when the patient was 5 months old. At 1 year of age, the patient underwent a cranial vault reshaping for raised ICP, proptosis, and aesthetic considerations, and at 3 years, a genioplasty was performed. Tonsillectomy and adenoidectomy were performed at 4 years of age as a result of upper airway restriction, and the patient underwent multiple myringotomies and grommet insertions.

Patient 2

This male patient with karyotype 46XY was delivered at 41 weeks' gestation by an emergency cesarean section for fetal distress. He suffered from intrauterine growth restriction, weighing 2.27 kg at birth and required intermittent positive-pressure ventilation and suction for meconium aspiration and was transferred to the special care baby unit.

On examination at birth, there was turriccephaly, midface hypoplasia and recession, a depressed nasal bridge, thick epicanthic folds, and retrognathia. There was a high-arched palate with fusion among the lower incisors and a class I skeletal relationship. Proptosis was present with a decrease in vision. The ears were low set and cupped with small thickened helices and bilateral moderate conductive hearing loss requiring a hearing aid. Although choanal stenosis was absent, the restricted upper airway resulted in severe OSAS. Expressive speech was found to be delayed on formal neuropsychologic assessment in later childhood.

There was significant restriction in pronation, supination, and extension of the forearms. The toes were long, and the great toes were broad with webbing of the first and second toes. There was impaired steroidogenesis with abnormalities in cortisol production.

The insertion of an ICP monitor demonstrated raised ICP at 4 months of age, and a cerebral angiogram at 1 year of age demonstrated abnormalities in intracranial venous drainage. Genetic screening revealed 2 separate mutations of the POR gene.

When the patient was 10 months old, a tonsillectomy and adenoidectomy were performed as a result of upper airway restriction. At 1 year of age, a posterior cranial vault expansion was performed for both ICP and aesthetic considerations. The patient underwent several myringotomies and grommet insertions during childhood.

Patient 3

This male patient with karyotype 46XY was delivered by an elective cesarean section. At birth, the cranial vault was turriccephalic with frontal bossing, and there was midface hypoplasia and recession. There was proptosis, resulting in severe exposure keratitis, hypertelorism with down-sloping palpebral fissures, and myopia of the right eye, and vision was further decreased secondary to optic atrophy. The palate was high and narrow, with a class III skeletal relationship and anterior open bite. The child had an OSAS. In later childhood, there was a global developmental delay on formal neuropsychologic assessment.

The classic feature of bilateral radiohumeral synostosis was present. The neck was stiff and straight with limited rotation, and radiographs revealed cervical spine fusion at the level of C1-C2 and multiple narrow intervertebral disk spaces thought to be the result of failure of segmentation during embryological development. There was a lumbar lordosis associated with a congenital anomaly of the lower dorsal vertebrae. Genetic screening revealed the S351C mutation of the FGFR2 gene. Hydrocephalus was present secondary to aqueductal insufficiency with increased ICP.

Before being seen at the craniofacial unit, the child had undergone both a fronto-orbital advancement (FOA) at 1 month of age and a ventriculoperitoneal (VP) shunt insertion at 2 months of age for proptosis, hydrocephalus, and raised ICP, and a bilateral tarsorrhaphy and a second FOA at 8 months of age in a further attempt to correct severe proptosis. A further FOA, palatal split, and bilateral tarsorrhaphies and canthopexies were subsequently performed at 2 years of age for ICP and aesthetic considerations, upper airway restriction, and proptosis. Further eye surgery was required, and at 5 years of age, a Le Fort III midfacial advancement was performed with distraction to correct the limited midfacial growth.

Patient 4

This female child with karyotype 46XX was delivered at 41 weeks' gestation by emergency cesarean section for cephalopelvic disproportion. There had been a diagnosis antenatally of probable Pfeiffer syndrome.

There was craniosynostosis of the coronal, lambdoid, and squamosal sutures resulting in the kleeblattschädel or cloverleaf deformity, as can be seen in Figure 1. There was midface hypoplasia and a beaked nose. The child demonstrated proptosis with hypertelorism, down-slanting palpebral fissures and a left ptosis, an esotropia, myopia, cataracts, and optic atrophy, all contributing to a decrease in vision. A class III skeletal relationship was present with an anterior open bite. The ears were low set with hearing loss requiring a hearing aid. There was a tendency to glossoptosis with a high-arched palate and delayed eruption of the permanent teeth. There was a severe speech and language delay on formal neuropsychologic assessment in later childhood, where expressive speech was affected to a greater degree. Upper airway obstruction was present with episodes of apnea.

There was bilateral radioulnar humeral synostosis. The thumbs were medially splayed with broad terminal phalanges. There was bilateral coxa vara with mild lateral subluxation and flexion deformities at the hips, knees, and ankles. The great toes were medially deviated, and the terminal phalanges were broad and irregularly shaped. The patient was wheelchair bound.

The neck was short, and radiology of the cervical spine revealed fusion at the level of C2-C3 and in the region of C5-C7, a midline split vertebral body at C3, a moderate scoliosis, and forward subluxation of C1 on C2 with narrowing of the spinal canal both in the cervical and upper thoracic regions. There was fusion of the spinous processes in the upper thoracic region and a mild upper lumbar gibbus.

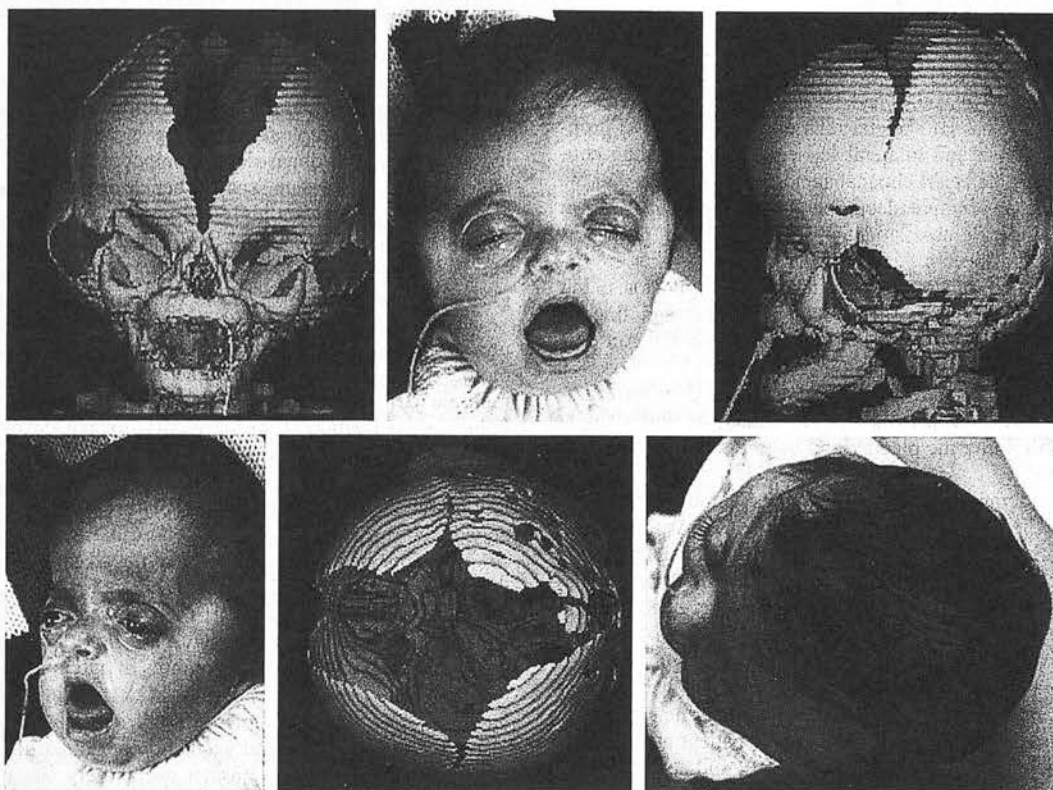


FIGURE 1. Three-dimensional computed tomography scan reconstructions and clinical photography of patient 4 demonstrating the kleeblattschädel or cloverleaf deformity.

Aqueductal obstruction was found on ultrasound at 1 month of age, resulting in hydrocephalus with increased ICP demonstrated with the insertion of an ICP monitor at 3 months of age. Genetic screening revealed the S351C mutation of the FGFR2 gene. A VP shunt was required as a result of hydrocephalus and raised ICP. A palatal split and adenoidectomy were performed at 1 month of age for upper airway restriction, and at 3 months of age, the patient underwent FOA and lambdoid craniectomies for raised ICP, proptosis, and aesthetic considerations, and a second palatal split procedure was performed. A further FOA was performed at 2 years of age, and a third FOA and cranial decompression were performed at 5 years. A Le Fort III midfacial advancement with distraction was performed at 11 years of age to correct the restriction in midfacial growth. Several grommet insertions were performed.

DISCUSSION

The diagnosis of ABS is primarily based on the presence of craniosynostosis and radiohumeral synostosis, that is, on clinical findings. However, the advent of genetic screening in relatively recent times has provided additional information on the pathogenesis of ABS, which is important for both researchers and clinicians. It is not surprising that an FGFR2 mutation has been identified in patients with ABS such as is known to occur in other craniosynostosis syndromes, a *de novo* heterozygous mutation of the FGFR2 gene resulting in the amino acid substitution S351C within the immunoglobulin-like domain III.³ However, despite clinical features suggestive of ABS, the S351C mutation of the FGFR2 gene was unable to be identified in an increasing number of cases, and it was noted not to occur particularly in those patients with genital anomalies and/or impaired steroidogenesis.¹⁴ Further investigation has revealed the common finding of several homozygous or

compound heterozygous mutations of the POR gene in many of those patients with ABS and abnormal genitalia and/or impaired steroidogenesis.^{13,15,16}

In the 2 patients described with ABS and abnormal genitalia and impaired steroidogenesis, mutations of the POR gene were present, and the S351C mutation of the FGFR2 gene was present in the 2 patients with normal genitalia and steroidogenesis. These 4 cases further demonstrate that within ABS there are 2 phenotypic groups with different underlying mutations of 2 separate genes with different modes of inheritance.

There have been several previously reported cases of ABS with hydrocephalus, and it is notable that in one of our cases, there was an Arnold-Chiari malformation.^{5,6,16-18} Patient 1 was found to have a type I Arnold-Chiari malformation, and both patients 3 and 4 were found to have aqueductal stenosis resulting in hydrocephalus and requiring a VP shunt. Patient 2 demonstrated raised ICP as the result of atypical abnormalities in intracranial venous drainage. We have been unable to find previous reports of these anomalies of the intracranial ventricular and venous systems within ABS.

The craniofacial morphology in ABS is often brachycephalic and when viewed from above may appear trapezoidal.^{1,4} Bilateral coronal synostosis occurs most frequently, whereas a smaller proportion of cases have lambdoid synostosis, and metopic synostosis is very rare.⁵ Patient 4 demonstrated coronal, lambdoid, and squamosal synostosis, resulting in the cloverleaf deformity, an indication of her more severe phenotype overall. However, given that the 4 patients in this series were aged beyond the neonatal period, the range of phenotypic severity may be reduced as a result of the high neonatal mortality rate within this syndrome.

Frontal bossing and a depressed nasal bridge with a "pear-shaped" nose are also features of ABS.^{4,17} The bony orbit is frequently shallow, resulting in proptosis, and there may be a recessed

and poorly defined supraorbital margin, which has been termed the "trumpet-bell orbital deformity."^{4,12} Visual loss is commonly multifactorial as is demonstrated by patients 3 and 4. The ears within ABS are typically low-set with a lop-ear appearance, and there may be both conductive and sensorineural hearing loss, in 2 of the above cases severe enough to require a hearing aid.^{1,11} Severe complex malocclusion has been reported in combination with crowded dentition, crossbite, open bite, and a narrow high-arched palate.^{1,17} A class III skeletal relationship was found in 3 of the above 4 cases, which is similar to that seen within other craniofacial syndromes; other features were a narrow high-arched palate, an anterior open bite, delayed eruption of permanent dentition, and fusion among the lower incisors. It was interesting to note that in all 4 of the above cases, speech and language were significantly affected and that in 3 of these patients expressive speech was affected to a greater degree, patient 4 being almost nonverbal.

All of the patients described in this study demonstrated significant upper airway obstruction, one with bilateral choanal stenosis requiring a tracheostomy for airway management. Although radiohumeral synostosis occurs most frequently and is generally considered to be one of the minimum diagnostic criteria of ABS, variability in elbow fusion does occur with radioulnar synostosis, radioulnar synostosis, and rare reported cases of ABS with absence of synostosis.^{3,6,7,9,16,19,20} Cases without radiohumeral synostosis may, however, represent atypical cases of other cranio-synostosis syndromes such as Pfeiffer syndrome. Bilateral elbow involvement is usual; however, there has been 1 reported case of unilateral radiohumeral synostosis.⁵ In our series, patients 1 and 4 demonstrated bilateral radioulnar synostosis.

Although craniosynostosis, radiohumeral synostosis, multiple joint contractures, and arachnodactyly are the most common skeletal findings within ABS, other variable features include femoral and ulnar bowing, perinatal fractures of the long bones, camptodactyly, carpal and tarsal bone synostosis, various abnormalities of the metatarsals and phalanges, talipes equinovarus, rocker-bottom feet, and vertebral anomalies of the thoracic and lumbar spine including fusions and spinal dysraphism.^{1,3,4,6,8,11,16} Interestingly, both patients 3 and 4 had cervical spine fusion, patient 3 at the level of C1-C2 and patient 4 involving C2-C3 and also C5-C7 with a forward subluxation of C1 on C2. The skeletal anomalies and subsequent deformity of patient 4 were in fact very severe, necessitating the use of a wheelchair. The vertebral bodies of patients with ABS have been reported to be of increased height, and the endplates sclerotic; clefts and intersegmental fusions of the thoracic and lumbar spine have also been reported.^{3,9} However, we have been unable to find previous reports of cervical spine fusion or subluxation within ABS. In the general population, cervical spine fusion is known to occur at an incidence of approximately 0.5% and usually occurs at the level of C2-C3.^{21,22} Cervical spine fusion occurs at a higher incidence within the acrocephalosyndactyly syndromes; within Pfeiffer syndrome, fusion occurs most frequently at C2-C3, whereas within Apert syndrome, fusion occurs most frequently at C5-C6; C1-C2 subluxation has also been reported to occur within the acrocephalosyndactyly syndromes.²²⁻²⁷ The cervical spine fusion seen in patients 3 and 4 does not seem to conform to a specific pattern such as is seen within these acrocephalosyndactyly syndromes. Cervical spine fusion may contribute to airway compromise and dysmorphic facial growth.²³

Surgical procedures in patients with ABS are primarily aimed at reducing hydrocephalus and raised ICP and correcting cranial shape, achieving orbital competency and addressing the limited midfacial growth. Consequently, the above patients underwent suture craniectomies or cranial vault reshaping, and 2 of the patients underwent FOA and midfacial advancement with distraction. As a result of significant upper airway obstruction, procedures such

as tonsillectomy and adenoidectomy, in 1 case, a tracheostomy, and in another, a palatal split, were performed in infancy or early childhood.

CONCLUSIONS

There is a wide spectrum of anomalies seen within ABS, and the severity of these anomalies is largely variable. There are 2 genotypic groups with underlying mutations of 2 separate genes and considerable overlap in the resulting phenotype. The distinguishing phenotypic feature seems to be the presence or absence of genital anomalies and/or impaired steroidogenesis. In the 2 patients in this series with genital anomalies and impaired steroidogenesis, mutations of the POR gene were present, and the S351C mutation of the FGFR2 gene was present in the remaining 2 patients with normal genitalia and steroidogenesis.

In this study, we report new anomalies in 4 cases of ABS that we believe have not been previously reported in the literature. Atypical intracranial anomalies were present in the ventricular and venous systems in 3 patients, cervical spine fusion was seen in 2 cases, and in one of these patients, there was also subluxation of the high cervical spine. Patient 4 demonstrated a very severe phenotype: the cloverleaf deformity was present, there was extensive musculoskeletal involvement resulting in the patient being restricted to a wheelchair, and the patient was almost nonverbal. Additional studies are required to further explore the differences in the phenotypic features of the 2 known genotypes within ABS.

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Anomalous venous drainage in a case of non-syndromic craniosynostosis

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Abstract The authors describe the clinical and radiological findings in a case of non-syndromic craniosynostosis affecting multiple sutures, in which the intracranial venous drainage was grossly anomalous. Investigation by magnetic resonance imaging and angiography revealed that almost all of the intracranial venous blood was draining from the dural sinuses transosseously via enlarged emissary veins to the external jugular veins and the vertebral veins.

Although present, both internal jugular veins were small. This discovery represented a contraindication for the vault expansion surgery that had been recommended as treatment for the patient's raised intracranial pressure, and it has important implications for the management of all types of craniosynostosis.

Key words Anomalous venous drainage · Craniosynostosis

Introduction

The factors influencing the surgical management of the patient with craniosynostosis include the degree of cosmetic disability, the presence or absence of raised intracranial pressure, the status of the airway and protection of the eyes. In the absence of active hydrocephalus and an obstructed airway, raised intracranial pressure is usually treated in our unit by a procedure designed to increase the intracranial volume and at the same time improve the patient's head shape [9].

We have previously reported the association between abnormal venous drainage and the severe skull base deformity seen in a patient with a clover-leaf skull [11]. In the case in question, the rapid rise in intracranial pressure that occurred when osseous to subcutaneous collateral venous channels were interrupted during surgery was thought to be an important factor in the patient's death [10]. Clearly, the possibility of anomalous venous drainage needs to be carefully assessed, and recommendations for cases of syndromic craniosynostosis have already been made [10].

The case report presented here describes the investigation of a child with non-syndromic craniosynostosis affecting multiple sutures, who was found to have anomalous venous drainage malformation in association with a large head and raised intracranial pressure.

Case report

An 8-month-old boy with an enlarged and asymmetric skull was referred to our Craniofacial Unit for assessment. He had been born to normal parents by caesarian section 2 weeks post-term following a normal pregnancy. At the time of referral he was developing normally and thriving. On examination he was noted to have marked frontal bossing, a small asymmetric anterior fontanelle and a left-sided posterior plagiocephaly (Fig. 1). Head circumference was measured and found to lie on the 98th centile. Anterior-posterior and lateral skull radiographs (Figs. 2, 3) helped us to establish a working diagnosis of left lambdoidal and partial sagittal synostosis. Early multidisciplinary in-patient craniofacial assessment was arranged.

As part of the patient's assessment, the radiologists confirmed the multiple suture nature of the craniosynostosis. The geneticists confirmed that this was a non-syndromic case. Psychological assess-

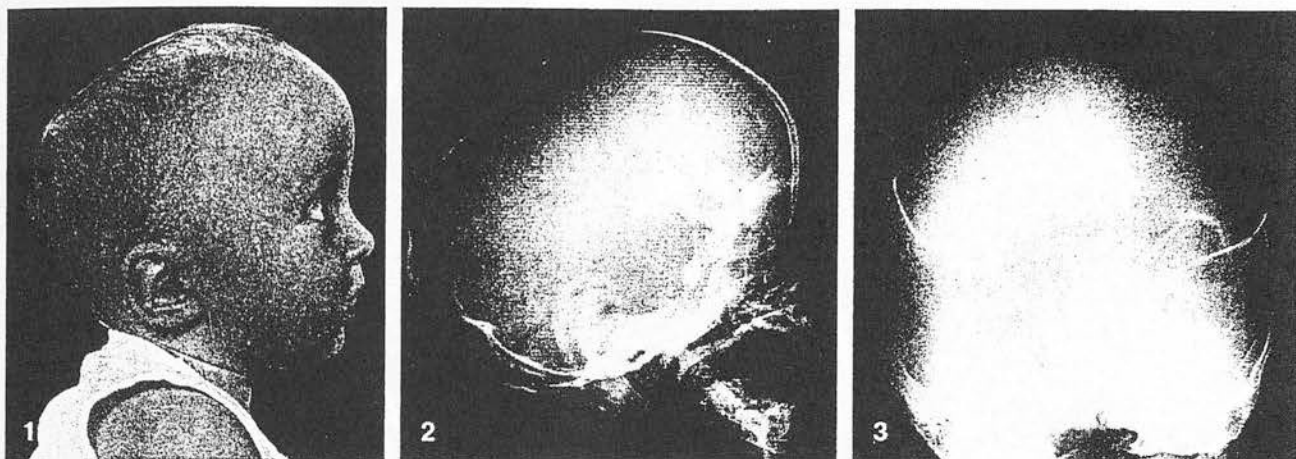


Fig. 1 Lateral photograph showing the enlarged appearance of the head at the time of first presentation

Figs. 2, 3 Skull radiographs demonstrating the unusual head shape (scaphocephalic with frontal bossing)

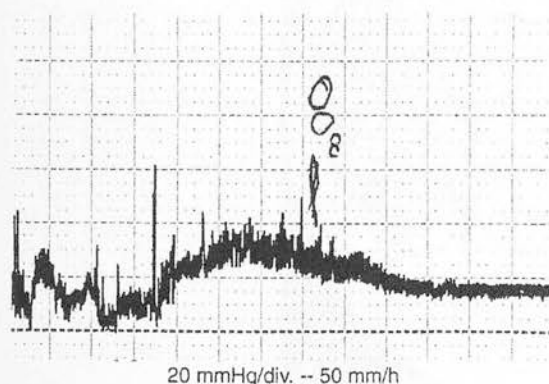


Fig. 4 Section of the intracranial pressure monitoring trace showing an elevated pressure plateau at 25 mmHg during sleep

ment showed that his development was appropriate for the boy's age. He underwent overnight intracranial pressure monitoring using the Camino fibreoptic system (Camino Laboratories, San Diego, Calif.). This revealed double plateaux of raised pressure, with a mean of 17.8 mmHg, and a maximum of 28 mmHg (Fig. 4).

Following this assessment, arrangements were made for early elective craniectomy of the fused sutures and vault remodelling. It was planned that this should be performed in two stages, starting with a posterior remodelling.

However, once the patient had been anaesthetised and placed prone on the operating table, a thrill could be palpated in the occipital region bilaterally. Auscultation of these areas revealed a loud venous "hum". Since this was thought to represent an arterial-venous malformation, the procedure was abandoned and arrangements made for further imaging to evaluate the cranial vascular anatomy.

Radiological findings

Magnetic resonance imaging (MRI) revealed that two large, anomalous blood vessels were present extracranially in the subcutaneous tissues, one on each side (Figs. 5, 6). However, as their function was unclear and the direction of any flow uncertain, we proceeded to angiography for more accurate assessment of the pattern of venous drainage of the cerebral circulation.

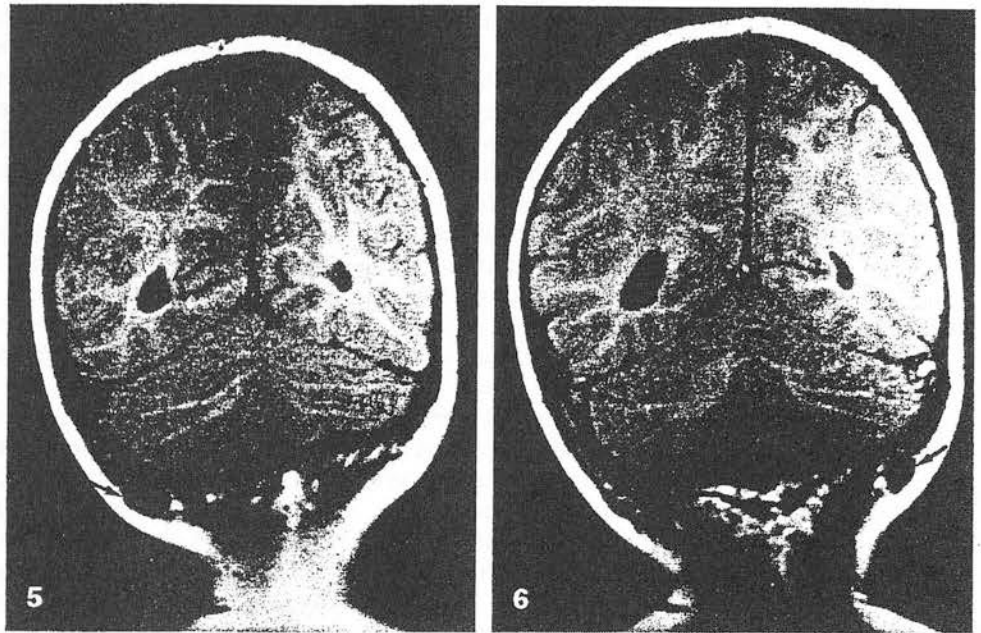
Carotid angiography revealed grossly abnormal extra-cerebral venous drainage (Figs. 7, 8). The internal jugular veins, although present, were small and appeared only to be filled by veins around the foramen magnum. On the left side the transverse sinus drained transosseously into the left external jugular vein. On the right side the sigmoid sinus was narrow but patent, the majority of the drainage being via large transosseous vessels running into the external jugular vein and the vertebral venous plexus. Other anomalous features included drainage of the right superficial temporal veins into the right transverse sinus and transosseous drainage of the sagittal sinus anteriorly into the facial vein.

In view of these findings, and taking into account the child's normal developmental status, it was felt that treatment of the child's raised intracranial pressure by vault expansion surgery was contraindicated. He remains under regular review, continuing to thrive, and there has been no deterioration in his appearance.

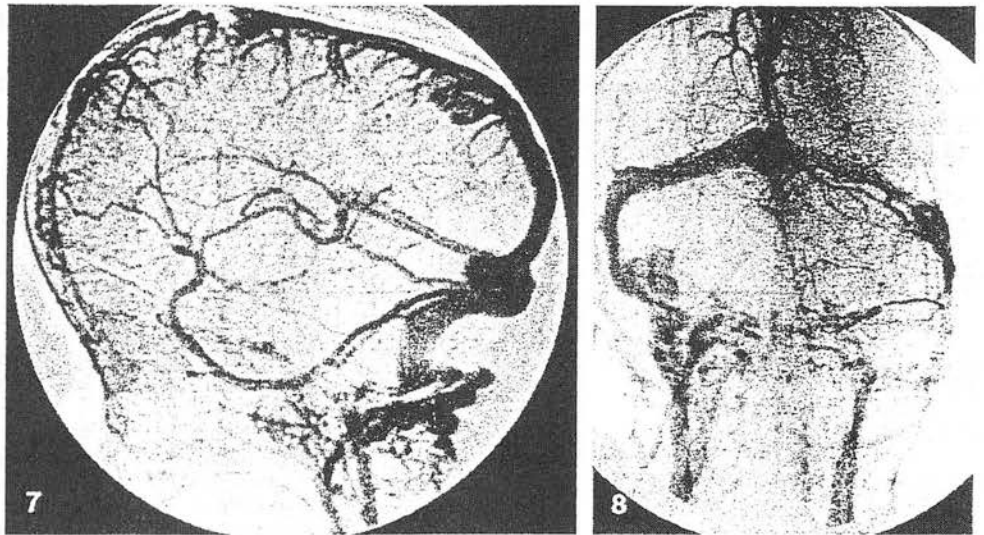
Discussion

The normal anatomy of the intracerebral veins and their extracranial drainage in man has been well described [1-3]. However, their development is complicated [12]. The primary capillary plexus of the early embryo develops into three layers following differentiation of the skull and me-

Figs. 5, 6 Magnetic resonance imaging scans in coronal section, showing the bilateral anomalous vessels (arrowed) lying extracranially



Figs. 7, 8 Sagittal and coronal views of the venous phase of the carotid angiogram, demonstrating the abnormal intracerebral venous drainage



ninges. The superficial vessels drain into the external jugular system. The middle vessels drain with the deep vessels via the internal jugular vein and also develop into the venous plexus of the dura mater from which the dural sinuses differentiate. Normally, connections between vessels of the superficial and middle layers persist variably as small emissary veins [12]. The veins that develop in the occipito-capsular fissure become the mastoid emissary veins in man [3]. The usual size of the mastoid emissary vein has been reported as 1 mm, with 10% over 2 mm in size, and only exceptional ones as much as 4 mm [1]. Reduction of the size of the sigmoid sinus has been noted to be associated with an enlargement of the mastoid emissary vein up to 7 mm in diameter [2]. It has been suggested that

wherever there are gaps in the developing chondrocranium there is a potential for connections between the developing dural sinuses and the external jugular system [3].

The embryological development of this area is therefore further complicated by the process of ossification of both the occipital and mastoid bones through which these emissary veins pass [8], making the incidence and position of their foramina in adult human populations particularly variable [3].

The development of the intracranial and extracranial venous systems and the flow of blood within them is also influenced by the position of the head in relation to the right atrium [5]. The vertebral venous system in the normal newborn child is functionally inadequate to act as a collateral

circulation to the jugular veins [5]. As the child's locomotor development progresses the head position changes from the horizontal position during crawling to the vertical for walking, and the functional anatomy of the vertebral venous system also evolves during this process [5].

An assessment of the anatomy of the venous system in patients with craniosynostosis can be made in several different ways. Plain radiography can be used to demonstrate emissary veins, a Towne's view being essential for those in the occipital region [4]. A review of MRI in the assessment of craniosynostosis with a discussion of how this can be related to improvements in clinical management has previously been reported [6]. However, although many uses were described, including assessment of the airway, hindbrain herniation and abnormalities of white matter, there was no reference to the detection of anomalous venous drainage, although its role in depicting the structure and function of the intracranial vessels is now well described [7]. Cerebral angiography provides the most accurate anatomical information regarding the anomalous vessels, confirming that they are indeed venous and demonstrating whether they are the main route of drainage of the dural sinuses.

It is unclear whether the craniosynostosis that resulted in the enlarged dysmorphic head shape directly influenced the head position and then led to the development of the

anomalous venous drainage seen in this case, or whether the raised intracranial pressure contributed. The presence of anomalous venous drainage in syndromic craniosynostosis has previously been reported [10, 11], including a case report in which large emissary veins in the occipital region were divided during surgery, with fatal consequences [10]. This highlights the clinical significance of the finding of this anomalous venous drainage, and so currently represents a contraindication for elective surgery in the presence of normal development.

Our experience with this case leads us to make the following recommendations for assessment of cases of craniosynostosis. First, to be alert of the possibility of abnormal venous drainage in all cases of craniosynostosis, and to study carefully both the plain radiographs and MRI scans for venous channels. Secondly, to remember that if abnormal venous drainage is found then it may be associated with raised intracranial pressure, and the consequences of interrupting this during surgery must be carefully considered.

In summary, this case demonstrates that anomalous venous drainage can occur in non-syndromic craniosynostosis. The anomalous venous drainage can be accurately assessed both anatomically and functionally by imaging. These findings may alter the clinical management of the patient, as this case illustrates.

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moderate or severe glaucoma. In addition, it cannot be excluded that the 24-hour rhythm of aqueous melatonin differs in glaucoma patients and in normal subjects. To confirm this possibility, further studies are needed in nonhuman primates over a 24-hour cycle. The reduction of IOP^{5,6} could possibly be mediated via melatonin receptors (as MT₃ receptor) located in the eye, in the anterior segment,⁷ or in the ciliary body.⁵

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Ophthalmic Findings in Apert Syndrome Prior to Craniofacial Surgery

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PURPOSE: To determine ophthalmic findings in patients with Apert syndrome before craniofacial surgery.

DESIGN: A cross-sectional retrospective study.

METHODS: Review of 63 cases (27 males, 36 females) with Apert syndrome without craniofacial surgery from the Australian Craniofacial Unit. Demographic data, age of presentation, and ophthalmic findings at the first presentation were recorded.

RESULTS: At a mean age of four years and median age of one year, at least 14% of patients had amblyopia, 60% of patients had strabismus, 19% of patients had anisometropia, and 34% of eyes had ametropia. Exposure keratopathy and corneal scarring occurred in at least 13% of patients and optic atrophy in at least 8% of patients.

CONCLUSIONS: This study demonstrated that patients with Apert syndrome were at risk of amblyopia because of high prevalence of refractive errors, strabismus, and anisometropia. Exposure keratopathy and corneal scarring occurred commonly. (*Am J Ophthalmol* 2006;142: 328-330. © 2006 by Elsevier Inc. All rights reserved.)

APERT SYNDROME IS A WELL RECOGNIZED CRANIOSYNOSTOSIS syndrome characterized by craniosynostosis, midfacial hypoplasia, and syndactyly of the hands and feet¹

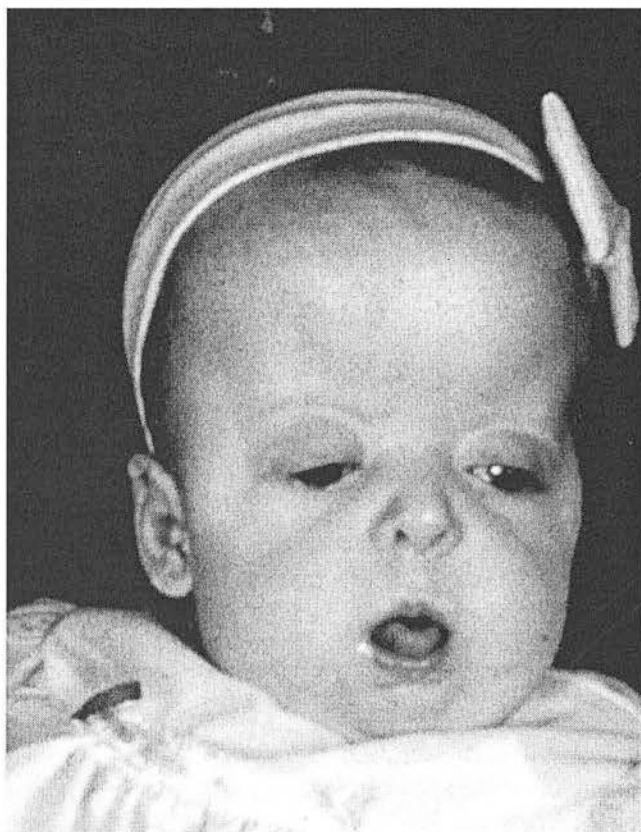


FIGURE 1. Preoperative features of Apert syndrome showing turricephaly, midface hypoplasia with shallow orbits, proptosis, down slanting palpebral fissure, and hypoplastic upper jaw with class III malocclusion.

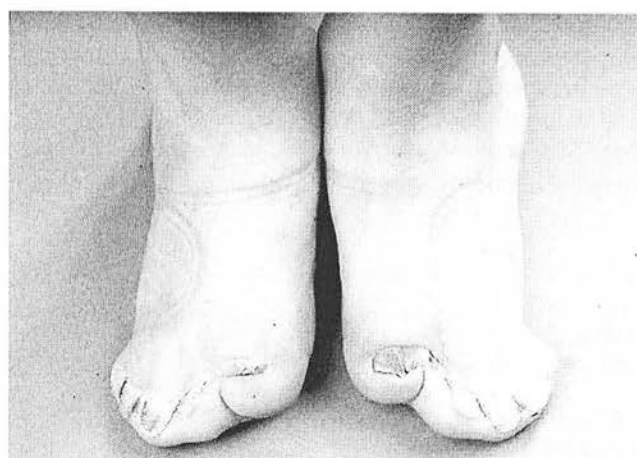
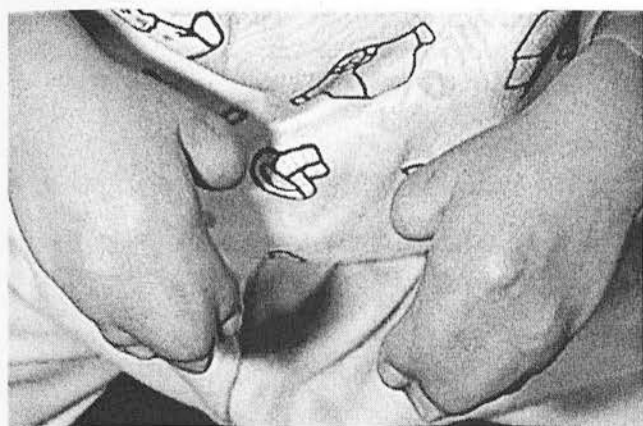


FIGURE 2. Syndactyly of the hands and feet is a universal finding in Apert syndrome. (Left) syndactyly of the hands. (Right) syndactyly of the feet.

TABLE. Ophthalmic Findings in Apert Syndrome at Presentation

Ophthalmic Findings	No. With Positive Finding (n)	Data Available	Occurrence as Percentages of Available Data	Occurrence as Percentages of Total (n = 63 cases = 126 eyes)
Visual impairment <6/12 in better eye	3	16 cases	3/16 (19%)	3/63 (5%)
Visual impairment <6/12 in at least one eye	7	16 cases	7/16 (44%)	7/63 (11%)
Refractive errors		88 eyes		
Hypermetropia	30		30/88 (34%)	30/126 (24%)
Myopia	13		13/88 (15%)	13/126 (10%)
Emmetropia	45		45/88 (51%)	45/126 (36%)
Astigmatism	37		37/88 (42%)	37/126 (29%)
Anisometropia	12	44 cases	12/44 (27%)	12/63 (19%)
Strabismus	38	58 cases	38/58 (66%)	38/63 (60%)
Type of strabismus				
Exotropia	21		21/58 (36%)	21/63 (33%)
Esotropia	11		11/58 (19%)	11/63 (17%)
Vertical	3		3/58 (5%)	3/63 (5%)
Exophoria	2		2/58 (3%)	2/63 (3%)
Manifest unspecified	1		1/58 (2%)	1/63 (2%)
Amblyopia	9	49 cases	9/49 (18%)	9/63 (14%)
Optic atrophy	5	60 cases	5/60 (8%)	5/63 (8%)
Optic disk swelling	3	60 cases	3/60 (5%)	3/63 (5%)
Abnormal VEP	9	42 cases	9/42 (21%)	9/63 (14%)
Corneal scar and exposure keratopathy	8	59 cases	8/59 (14%)	8/63 (13%)
Proptosis	55	60 cases	55/60 (92%)	55/63 (87%)
Ptosis	14	44 cases	14/44 (32%)	14/63 (22%)

VEP = visual evoked potentials.

(Figures 1 and 2). Visual loss is a severe complication of Apert syndrome, which could result from optic atrophy, amblyopia, and exposure corneal scarring.^{2,3} Absence or structural alteration of extraocular muscles, iris coloboma, cataract, ocular albinism, keratoconus, ectopia lentis, and medullated nerve fibers had also been reported with this syndrome.²⁻⁶ However, most publications either described

case reports or small series of Apert syndrome or findings of combined craniosynostoses. The aim of this study was to determine the prevalence of ophthalmic findings in Apert syndrome before craniofacial surgery in a large series of patients.

Retrospective reviews of 87 patients diagnosed with Apert syndrome from 1975 to 2004 were identified from

the Australian Craniofacial Unit database. Ethics approval was obtained from institutional review board taking into consideration that informed consent was not possible for patients living overseas. Patients who had craniofacial surgery before their first presentation were excluded. Sixty-three cases (27 males and 36 females) were therefore included in the study. The mean age at first presentation was four years (range 0.1 to 39.2 years, SD 8.21) and the median age was one year.

The parameters investigated included visual acuity, cycloplegic refraction, amblyopia, strabismus, anterior segment findings, fundus findings, and visual evoked potentials (VEP). Because of the retrospective nature of this study, not all data were present for all parameters: 16 notes contained Snellen visual acuity, 44 had data on cycloplegic refraction, 58 had data on strabismus, 49 had data on amblyopia, 59 had documented anterior segment findings, 60 had documented fundus findings, and 42 had data on VEP.

There were at least three patients (5%) with visual impairment worse than 6/12 in the better eye, and at least seven patients (11%) with visual impairment in at least one eye. At least nine patients (14%) had amblyopia. Strabismus was noted in at least 38 patients (60%) of the study population. Exotropia was more common than esotropia (21 patients compared with 11 patients).

At least 30 eyes (24%) had hypermetropia $\geq +2$ diopters (D), 13 eyes (10%) had myopia ≤ -0.5 diopters, and 37 eyes (29%) had astigmatism ≥ 0.75 diopters. In addition, at least 12 patients (19%) had anisometropia ≥ 0.75 diopters.

Fundoscopy revealed optic atrophy in at least five patients (8%), and optic disk edema in three patients (5%). At least nine patients (14%) had abnormal VEP, four bilaterally. Latency was prolonged in six cases while the amplitude was suppressed in three cases. Only two of the nine cases with VEP abnormality had optic atrophy and disk edema.

Proptosis was present in at least 55 patients (87%), and corneal scarring and exposure keratopathy were present in at least eight patients (13%). Ptosis (marginal reflex distance < 3 mm) was present in at least 14 patients (22%), two were unilateral, and 12 were bilateral (Table).

Other notable ophthalmic findings included bilateral myelinated optic nerve fibers (one), entropion (one), epiblepharon (two), trichiasis (two), coloboma of the disk (two), and tortuous retinal vessels (one).

This is the largest study reported looking at ophthalmic findings in Apert syndrome before craniofacial intervention. We found that patients with Apert Syndrome were at risk of amblyopia because of high prevalence of refractive errors, anisometropia, and strabismus. Our study is in agreement with earlier studies, which showed that exotropia was more common than esotropia in Apert syndrome.^{3,4} These patients need regular ophthalmic evaluation for early detection of amblyopia to institute

timely management. Our study also found that exposure keratopathy and corneal scarring occurred commonly. Invariably exposure keratopathy was attributable to inadequate lubrication and lid protection secondary to proptosis.^{2,3,7} The extent of visual impairment and amblyopia could be better assessed by a prospective study following the cohort through to visual maturation.

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Pigment Release and Secondary Glaucoma After Implantation of Single-piece Acrylic Intraocular Lenses in the Ciliary Sulcus

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PURPOSE: To report the association of sulcus-fixated, single-piece hydrophobic acrylic intraocular lenses (HAIOL) with pigment release and secondary glaucoma.

DESIGN: Interventional case series.

METHODS: HAIOL was implanted in the ciliary sulcus of 20 eyes that developed posterior capsule rupture during phacoemulsification. We analyzed postoperative best-corrected visual acuity, manifest refraction, frequency of intraocular lens malpositioning, and postoperative complications.

RESULTS: Postoperative best-corrected visual acuity was 20/40 or better in all eyes. The mean postoperative sphere was -0.5 ± 0.7 diopters (range $+1.25$ to -2.00); the mean postoperative cylinder was $-1.2 \pm$

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Differential Effects of *FGFR2* Mutation in Ophthalmic Findings in Apert Syndrome

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Apert syndrome is mostly caused by one of the two specific point mutations in the fibroblast growth factor receptor 2 (*FGFR2*). The objective of this study was to determine whether there were any differences in the prevalence of ophthalmic features in Apert syndrome when comparing the Ser252Trp and Pro253Arg mutations in *FGFR2*. This was a retrospective study of patients with Apert syndrome with genotype analysis. The prevalence of five ophthalmic features, visual impairment, amblyopia, strabismus, corneal abnormality, and pale optic discs, were compared between the two *FGFR2* genotypes. There were 25 (74%) cases with Ser252Trp mutation, and 9 (26%) cases with the Pro253Arg mutation in *FGFR2*. Ophthalmic findings in 20 cases of *FGFR2* Ser252Trp and 9 cases of Pro253Arg mutation were compared. Visual acuity worse than 6/12 in at least one eye was present in 60% patients with *FGFR2* Ser252Trp mutation compared with 12.5% patients with Pro253Arg mutation ($P < 0.05$). Forty percent of eyes with *FGFR2* Ser252Trp mutation compared with 12.5% eyes with Pro253Arg mutation were worse than 6/12. There was a trend of more frequent amblyopia and strabismus in *FGFR2* Ser252Trp mutation and more frequent optic disc pallor in the *FGFR2* Pro253Arg mutation. There was a differential effect of *FGFR2* mutations in ophthalmic findings in patients with Apert syndrome, with significantly greater prevalence of visual impair-

ment in the Ser252Trp mutation compared with the Pro253Arg mutation. Further study would elucidate whether the trends in differential effects between the two mutations in amblyopia, strabismus, and optic disc pallor represent real differences.

Key Words: Apert syndrome, *FGFR2* mutation, craniosynostosis, genotype

Apert syndrome is one of the most common and severe craniosynostosis syndromes and is characterized by craniosynostosis, midface hypoplasia and syndactyly of the hands and feet. Since the discovery of fibroblast growth factor receptor 2 (*FGFR2*) mutations being Ser252Trp or Pro253Arg in 1995,¹ geneticists and clinicians have tried to determine whether there is any phenotypic difference between the two mutations to understand the pathophysiology of Apert syndrome. The results thus far are contradictory.²⁻⁴ Some studies showed no significant difference in some of the phenotypic features between the two common forms of *FGFR2* mutations.^{2,5} This contrasts with other studies, which showed that syndactyly was more severe and mental outcome was poorer in Pro253Arg mutation.^{3,4,6} Cleft palate and marked craniofacial malformation were found more commonly in the *FGFR2* Ser252Trp mutation.^{3,4,6}

Visual impairment and amblyopia were common findings in patients with Apert syndrome.⁷ Severe visual loss associated with papilledema had also been reported.⁸ Our aim was to further investigate whether there were any differences in the prevalence and severity of ophthalmic features between the Ser252Trp and Pro253Arg mutations in *FGFR2*.

MATERIALS AND METHODS

All cases of Apert syndrome were identified from the Australian Craniofacial Unit database. Only patients who had mutation analysis were included in the study.

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Table 1. Phenotype Genotype Correlations in Patients With Apert Syndrome

	FGFR2 S252W		FGFR2 P253R		P Value
Total no. patients	20		9		
VA <6/12 in better eye	4 (n = 20)	20%	1 (n = 8)	12.5%	1.000
VA <6/12 in at least one eye	12 (n = 20)	60%	1 (n = 8)	12.5%	0.038
Pale optic disc	3 (n = 19)	16%	2 (n = 7)	29%	0.588
Amblyopia	10 (n = 18)	56%	1 (n = 5)	20%	0.339
Total no. eyes	40		18		
VA <6/12 (individual eyes)	16 (n = 40)	40%	2 (n = 16)	12.5%	0.061
Corneal scarring and keratopathy (individual eyes)	7 (n = 32)	25%	3 (n = 14)	21%	1.000
Strabismus (individual eyes)	18 (n = 38)	47%	7 (n = 18)	39%	0.546

FGFR = fibroblast growth factor receptor; VA = visual acuity.

The following variables were recorded: patient demographic data such as sex and age of ophthalmic review. Preoperative finding of strabismus was recorded. Visual acuity, presence of amblyopia, corneal scarring, keratopathy, and pale optic disc were determined at the last ophthalmic review. The prevalence of five ophthalmic features, ie visual impairment, amblyopia, strabismus, corneal abnormality, and pale optic discs, and the two *FGFR2* mutations Ser252Trp and Pro253Arg were compared. Cases with fewer than three recorded variables were excluded from the study.

Best corrected visual acuity was measured by methods appropriate to age and verbal capacity using Snellen chart, Kay pictures, and Sheridan Gardiner tests and were recorded as Snellen or Snellen equivalent. In preverbal children, fixation pattern was determined as fixing and following. Visual impairment was defined as having a visual acuity of worse than 6/12.

Statistics were analyzed using Minitab version 14 (Minitab, Inc., State College, PA). Two proportions tests were performed to compare the differences between the prevalence of each ophthalmic finding for the two genotypes. Fisher's exact test is used to account for the small sample size. $P < 0.05$ was considered statistically significant.

RESULTS

Thirty-four cases of Apert syndrome had results of genetic testing available for assessment, which included 19 males and 15 females. There were 25 (74%) cases with the Ser252Trp mutation and 9 (26%) cases with the Pro253Arg mutation in *FGFR2*. Five cases were excluded because of insufficient ophthalmic data, and all had the *FGFR2* Ser252Trp mutation. Therefore, ophthalmic findings of 20 cases of *FGFR2* Ser252Trp and 9 cases with the *FGFR2* Pro253Arg mutation were analyzed. The average age of the last

ophthalmic review for the *FGFR2* Ser252Trp and *FGFR2* Pro253Arg mutations was 9.4 years and 13.1 years, respectively.

The prevalence of visual impairment in at least one eye in patients with Apert syndrome was greater in the *FGFR2* Ser252Trp mutation compared with the *FGFR2* Pro253Arg mutation, and the result was statistically significant. Visual acuity worse than 6/12 in at least one eye was present in 60% of *FGFR2* Ser252Trp mutations compared with 12.5% in the Pro253Arg mutations (95% confidence interval [CI], 0.16–0.79, Fisher's exact test P value = 0.038). When comparing the number of eyes with visual impairment in the two Apert subgroups, 40% of eyes in the *FGFR2* Ser252Trp subgroup compared with 12.5% of the Pro253Arg subgroup were visually impaired (95% CI, 0.05–0.50, Fisher's exact test P value = 0.061), and the result was of borderline statistical significance. Amblyopia and strabismus were more frequent in *FGFR2* Ser252Trp subgroup than in *FGFR2* Pro253Arg subgroup, but the differences did not approach statistical significance. Pale optic discs were observed more with the *FGFR2* Pro253Arg mutations than with the *FGFR2* Ser252Trp mutations; however, the subject numbers were small, and the results were not statistically significant. There was no significant difference in the prevalence of corneal abnormality (corneal scarring and exposure keratopathy) between the two genotypes. The phenotype and genotype correlations of ophthalmic features are summarized in Table 1.

DISCUSSION

Apert syndrome is an autosomal dominant disorder that is distinguished from other craniosynostotic syndromes by the remarkably specific mutations of either Ser252Trp or Pro253Arg in the *FGFR2* gene on chromosome 10q26.¹ The two mutations occur in the linker region between

immunoglobulin II and immunoglobulin III of the *FGFR2* ligand binding domain.¹ The two mutations are exclusively paternal in origin.⁹ A gain of function in the mutant *FGFR2* was proposed to be the molecular mechanism of *FGFR2* mutation.^{10,11} It was shown that Ser252Trp mutation and Pro253Arg mutation in *FGFR2* led to enhanced affinity of *FGFR2* to ligands such as the FGF2 and other fibroblast growth factors.¹⁰ Interestingly, loss of ligand-binding specificity of mutant Pro253Arg *FGFR2* has also been reported.¹¹ The differential downstream effects of the two common mutations resulting in Apert syndrome are yet to be fully determined, and it remains a subject of great interest. The investigations of the differential effects of the two mutations in Apert syndrome have implications for the understanding of the pathophysiology of congenital malformations in *FGFR2* mutation. The proportion of the two *FGFR2* mutations in our population of Apert patients were 74% for Ser252Trp mutation and 26% for Pro253Arg mutation, which was similar to other studies looking at genotype-phenotype correlations for the two most common Apert syndrome genotypes.²⁻⁴

Clinical variability of the phenotypic features in Apert syndrome is well documented. The severity of syndactyly and the extent of craniofacial malformation and other systemic malformations involving the central nervous system, musculoskeletal, cardiac, and urogenital systems vary among individuals affected by Apert syndrome.^{2,3} Analysis of the two common mutations *FGFR2* Ser252Trp and Pro253Arg showed that patients with the Ser252Trp mutation presented more often with cleft palate and had more severe postoperative midface retrusion, which suggested that craniofacial malformation was more severe in the Ser252Trp mutation.^{3,4,6,11} Conversely, the *FGFR2* Pro253Arg mutation was associated with more severe syndactyly.^{3,4,6,11} The degree of intellectual disability also appeared to be worse in the *FGFR2* Pro253Arg mutation subgroup.⁴ However, no significant differences in clinical features were found between the two genotypes in an earlier study.²

The correlation of phenotypic features to genotypes in our study showed that there was a significant difference in visual outcome in patients with the Ser252Trp and Pro253Arg mutations in *FGFR2* at the last ophthalmic review. Patients with the *FGFR2* Ser252Trp mutations more often had visual impairment worse than 6/12 in at least one eye compared with patients with the *FGFR2* Pro253Arg mutations. The finding of worse visual outcome might relate to more severe craniofacial malforma-

tions in the *FGFR2* Ser252Trp mutations noted in previous studies.^{3,4,6,11} Fusion of multiple cranial sutures restricts intracranial and orbital space expansion in Apert syndrome. The orbits become lateralized and shallow, which results in proptosis with exposure of the globes, strabismus, and hypertelorism.^{12,13} However, it was not possible to determine from this study the reasons behind the difference in the prevalence of visual impairment of the two genotypes because of a small sample size. There was suggestion from this study that the difference in the prevalence of visual impairment between the two genotypes may be related to the more frequent findings of amblyopia and strabismus in the *FGFR2* Ser252Trp mutation. The power of the findings in this study was limited by the small number of patients. In consideration of the rarity of Apert syndrome, combined data from multiple craniofacial units would be an alternative to further evaluate the differential effects of *FGFR2* Ser252Trp and Pro253Arg on the ophthalmic findings.

In conclusion, there was evidence of a differential effect of *FGFR2* mutations in ophthalmic findings in patients with Apert syndrome, with significantly greater numbers of patients with visual impairment in the *FGFR2* Ser252Trp mutation compared with the Pro253Arg mutation. Although there was a trend of more frequent amblyopia and strabismus in the Ser252Trp mutation and more frequent optic disc pallor in the Pro253Arg mutation in *FGFR2*, the results did not reach statistical significance. Further study would elucidate whether these trends represent a real difference.

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Pyrexia after Transcranial Surgery

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Abstract: Pyrexia after transcranial surgery has been observed regularly in clinical practice but does not usually herald any subsequent pathologic process. However, the significance and incidence of this phenomenon remain uncertain. The aim of this study was to evaluate the incidence and timing of any pyrexia after transcranial surgery for craniosynostosis correction and correlate this with the clinical outcome to assess its significance. Retrospective review of sequential case notes collected over a 10 year period identified 136 transcranial operations undertaken for 122 cases of nonsyndromic craniosynostosis. The incidence of postoperative pyrexia of 38 degrees or more in the first 5 days was 76%, whereas that greater than 39 degrees was 11%. Pyrexia was noticed during the first 48 hours and had a bimodal distribution. Only a single case in this series subsequently developed a clinically significant complication, that is, a minor wound infection of the skin, which was treated by antibiotics and dressings. The occurrence of pyrexia did not appear to be related either to sex or to any affected suture but occurred less frequently in those who were under 6 months old. We conclude that this pyrexia should be considered to be a part of the normal physiological response to craniofacial surgery.

Key Words: Craniosynostosis, pyrexia

After transcranial surgical correction of nonsyndromic craniosynostosis, we have observed in the early postoperative phase that patients frequently develop a pyrexia, but the clinical impression has been that many of patients do not have any subsequent adverse clinical outcome. Review of the literature identified previous reports stating that this phenomenon is common after other types of surgical interventions, without any subsequent pathologic significance.¹⁻⁶

This contrasts with the findings that postoperative pyrexia is well recognized to be a predictor of infection.¹ It has also been observed during the acute phase of various neurosurgical traumas, including subarachnoid hemorrhage,⁷ intracerebral hemorrhage,⁸ or traumatic brain injury,⁹ in which development of

pyrexia is a poor prognostic sign.¹⁰ Central nervous system tissue appears to be more temperature labile than other body tissues, suggesting that pyrexia after transcranial surgery may not have the same underlying mechanisms as that after abdominal surgery.^{11,12}

To evaluate the incidence, significance, and subsequent clinical outcome of pyrexia after transcranial surgery, it was decided to undertake retrospective review of the postoperative temperature charts and clinical notes of a consecutive series of children with nonsyndromic craniosynostosis undergoing transcranial correction.

METHOD

With use of the database of the Australian Craniofacial Unit, those who underwent transcranial correction of nonsyndromic craniosynostosis in the 10 years between 1994 and 2003 were identified. The case notes were reviewed, including the postoperative temperature charts. Temperature charts consisted of a preoperative temperature, every 3 hour recordings in the postoperative period for 48 hours, and every 12 hour recordings for another 3 days. Independent variables that were studied include patients' sex, age, affected suture, and recorded postoperative complications.

RESULTS

The unit database identified 122 patients with nonsyndromic craniosynostosis who underwent transcranial surgery during the 10 years from 1994 to 2003 in which all records were complete to allow for evaluation. The population consisted of 77 males and 45 females. The patients' age at operation ranged between 2 months and 15 years, with a mean of 16 months. Among 136 operations, 66 were for patients aged under 6 months, and 44 were for those aged between 6 and 24 months. The cases were categorized according to the affected suture: sagittal synostosis

Table 1. Number of Corrective Transcranial Surgeries Performed to Each Type of Nonsyndromic Craniosynostosis

Affected Suture	No. of Cases	No. of Surgeries
Sagittal	52	61
Coronal	31	35
Metopic	25	25
Multiple	12	13
Lambdoid	2	2
Total	122	136

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Table 2. Number of Patients with Pyrexia after Surgery

Temperature	Time After Surgery					
	Within 12 Hours	Day 1	Day 2	Day 3	Day 4	Day 5
$T < 38$	98	73	72	112	122	118
$38 \leq T < 39$	34	58	60	24	14	18
$39 \leq T$	4	5	4	0	0	0

T = highest body temperature in each period.

was the most common and consisted of 52 cases, with unicoronal synostosis in 31 cases and metopic synostosis in 25 cases. Thirteen of 122 patients underwent multiple surgical interventions resulting in 122 patients undergoing a total of 136 transcranial operations (Table 1). Intraoperatively, the length of surgical intervention was between 21 minutes and 4 hours and 5 minutes (mean 2 hr and 1 min). When compared among affected suture groups, the shortest operating times were in the sagittal synostosis group. Broad spectrum antibiotics were administered intravenously as a single perioperative dose. All patients required transfusion perioperatively.

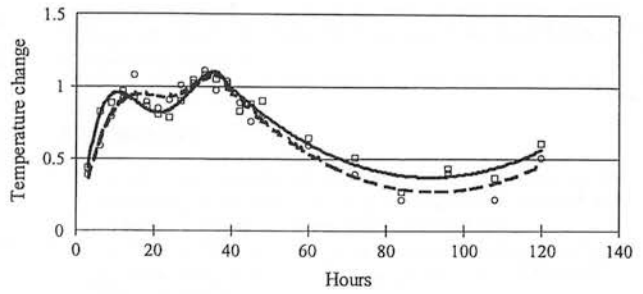
Postoperatively, patients were managed according to the unit protocol and were transferred to the intensive care unit (ICU) for at least 24 hours. There, they were nursed head-up at least 45 degrees to prevent swelling. Prescribed analgesia was paracetamol and narcotics were rarely used. In 103 of 136 patients, development of pyrexia was 38 degrees or higher during the first 5 postoperative days, and in 11 patients, it was 39 degrees or higher. Day 2 was the most common period for pyrexia, with 64 patients affected (47%) (Table 2). Analysis of the results revealed that there was no obvious difference between sexes or which suture was affected (Table 3). There was no correlation with the white blood cell count or length of ICU stay.

For each case, a chart of postoperative temperature change starting with the preoperative tem-

Table 3. Incidence of Postoperative Pyrexia in Each Type of Craniosynostosis

Affected Suture	No. of Febrile Cases and Each Percentages	$38 \leq T < 39$	$39 \leq T$
Sagittal	45	74	40
Coronal	25	71	22
Metopic	20	80	19
Multiple	11	85	9
Lambdoid	2	100	2
Total	103	76	92

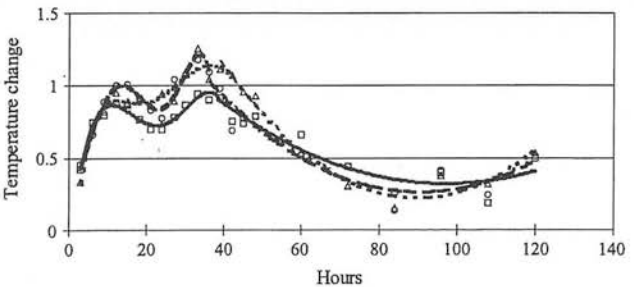
T = highest postoperative temperature during 5 days.

**Fig 1** Postoperative temperature course by sex. Square mark and solid line, male; round mark and dotted line, female.

perature was made and compared. The mean temperature changes in each group of sexes, affected suture, and age at operation were plotted on a graph, and a trend line was drawn with Microsoft Excel (Redmond, WA). In general, the postoperative temperature courses revealed a curious double peak in the first 48 hours, which was independent of sex, suture, and age (Figs 1 to 3). There was no sex difference (Fig 1). Interestingly, the temperature rise was slightly less in the group of sagittal synostosis than either coronal or metopic synostosis (Fig 2). Overall, the pyrexia was less marked in those patients whose surgery was undertaken when they were under 6 months (Fig 3).

DISCUSSION

Pyrexia is a feature of the inflammatory processes and is one of the most common signs of postoperative complication, particularly infection. The pyrexia is produced by bacterial endotoxins or exotoxins, which stimulate phagocytic leukocytes of the infected host to produce a series of endogenous pyrogens, producing pyrexia.¹³ These same endogenous pyrogens are also active after trauma, including surgery, in the absence of infection. Only one patient in this series

**Fig 2** Postoperative temperature course by affected suture. Square mark and solid line, sagittal; round mark and heavy broken line, coronal; triangular mark and dotted line, metopic synostosis.

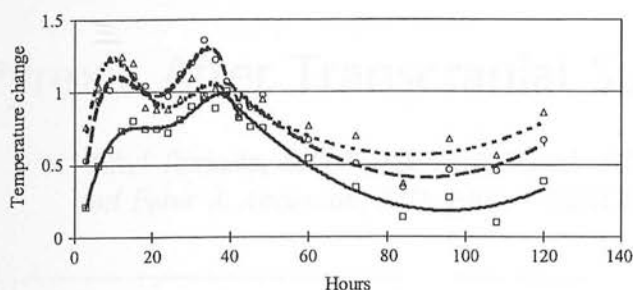


Fig 3 Postoperative temperature course by age at operation. Square mark and solid line, under 6 months; round mark and heavy broken line, 6 to 24 months; triangular mark and dotted line, over 24 months.

developed significant infection, and her temperature chart was unremarkable, although her infection was apparent on physical examination.

A previous study of craniofacial patients has reported the incidence of postoperative pyrexia to be 81%, which is similar to the 76% in our study. However, the bimodal distribution that we identified does not appear to have been previously reported among patients undergoing craniofacial surgery. Curiously, this has been noticed after cardiac surgery.¹⁴ The etiology of this double-peak phenomenon remains unclear, and more careful and divergent analysis is needed. We speculate that prospective measurement of known pyrogens,¹⁵ such as interleukin (IL)-1, tumor necrosis factor- α , IL-6, or interferon- γ , may assist in elucidating the cause, and we recognize that the two peaks might be caused by two different agents.

Finally, the correlation between the longer duration of surgery and the higher incidence of complication, including pyrexia, have been suggested by previous studies.^{16,17} This is highlighted in our study where the observed temperature rise was slightly less in the cases of sagittal synostosis than either coronal or metopic synostosis. We speculate that this might be caused by the differences in the length of their surgical intervention.

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Pyrexia After Transcranial Surgery for Pfeiffer Syndrome

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Background: Previously, we have reported the pattern of temperature increase after transcranial surgery for nonsyndromic craniosynostosis. It was found that pyrexia had a bimodal distribution during the first 48 hours after surgery.

Aim: The aims of this study were to evaluate pyrexia after transcranial surgery for syndromic craniosynostosis (Pfeiffer syndrome), to investigate whether the same pattern occurred, and to evaluate the correlation between pyrexia and possible factors, that is, sex, age, procedure, length of surgery, and incidence of postoperative cerebrospinal fluid (CSF) leakage.

Method: Twenty-one sequential case notes of Pfeiffer syndrome were retrospectively reviewed to collect 38 postoperative temperature courses. The mean change of temperature was plotted on a graph with a trend line to find the feature of the course.

Results: Pyrexia after transcranial surgery for Pfeiffer syndrome had a bimodal distribution during the first 48 hours, similar to the pyrexia after transcranial surgery for nonsyndromic craniosynostosis. This pyrexia was higher and more prolonged in those undergoing a longer surgical procedure and frontofacial advancement and procedures accompanied with postoperative CSF leakage. Moreover, the temperature course was more complex in procedures accompanied with postoperative CSF leakage.

Conclusions: It was concluded that in Pfeiffer syndrome, which has more complicated pathologic status than nonsyndromic craniosynostosis, also had bimodal postoperative temperature course. Although the etiology of the bimodal pyrexia remains unclear, it seems that it is part of the normal postoperative course in these cases. However, prolonged raised temperature within the first 48 postoperative hours may suggest a complication.

Key Words: Pyrexia, craniosynostosis, Pfeiffer syndrome

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Pyrexia is a commonly observed phenomenon in practice after any kind of surgery. Among the different reasons for fever origin, infection is recognized as one of the most common causes of pyrexia. Because infection can be life threatening, it is important not to overlook the infectious cause of pyrexia.^{1,2} However, it is a common clinical observation to encounter postoperative temperature increase without infection after surgery, and distinguishing fever resulting from surgical complication from a raised temperature after surgery is not always easy. Indeed, to distinguish infectious fever from normal postoperative pyrexia, some reports have identified the differences between infectious and noninfectious fevers.^{3,4}

To clarify features of pyrexia after transcranial surgery for craniosynostosis, we have previously reported the pattern of temperature increase after transcranial surgery for nonsyndromic craniosynostosis.⁵ It was found that temperature increase had a bimodal distribution during the first 48 hours after surgery. However, syndromic craniosynostosis has a different genetic pathology from nonsyndromic craniosynostosis.⁶ Moreover, syndromic craniosynostosis is usually accompanied with severe midface hypoplasia that requires midface advancement, and with additional surgery, it is possible that postoperative temperature course may have different features.

The aims of this study were to evaluate the temperature after transcranial surgery for syndromic craniosynostosis (Pfeiffer syndrome), to investigate whether the same pattern occurred, as in nonsyndromic craniosynostosis, and also to evaluate the correlation between pyrexia after transcranial surgery for Pfeiffer syndrome and possible factors, that is, sex, age, procedure, length of surgery, and incidence of postoperative cerebrospinal fluid (CSF) leakage.

METHOD

The database of the Australian Craniofacial Unit was used to identify patients with Pfeiffer syndrome who underwent transcranial surgery between 1985 and 2007. The records including postoperative temperature course were observed and recorded. The mean temperatures of each 4 hours within 48 hours and each 8 hours afterward, respectively, after the procedure were evaluated. The mean change of temperature was plotted on a graph with a trend line of a 3-period moving average, using Microsoft Excel to find the feature of the course.

RESULT

Twenty-one sequential case notes of Pfeiffer syndrome were retrospectively identified. The population consisted of 10 men and 11 women. Thirty-eight transcranial operations were performed on these 21 patients. Transcranial operations consisted of 25 fronto-orbital advancement with or without cranial reshaping, 6 frontofacial advancement with or without distraction osteogenesis, and 7 craniectomies (Table 1). Postoperatively, patients were managed according to the unit protocol. Prescribed analgesia was paracetamol, and narcotics were rarely used. There was no case that was complicated by an obvious surgical site infection that required surgical intervention or nonsurgical site infection such as a respiratory tract infection within the first 5-day postoperative period.

TABLE 1. Number of Patients Who Underwent Transcranial Surgery for Pfeiffer Syndrome

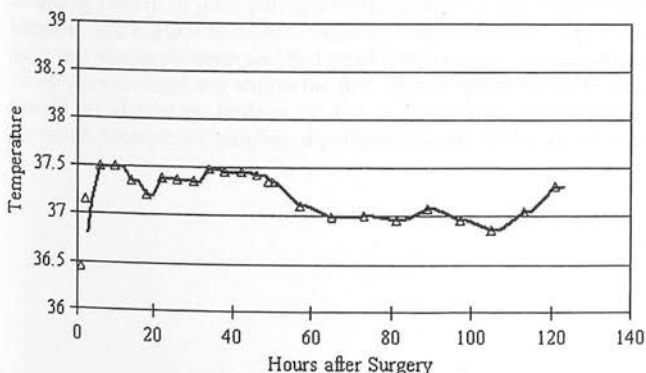
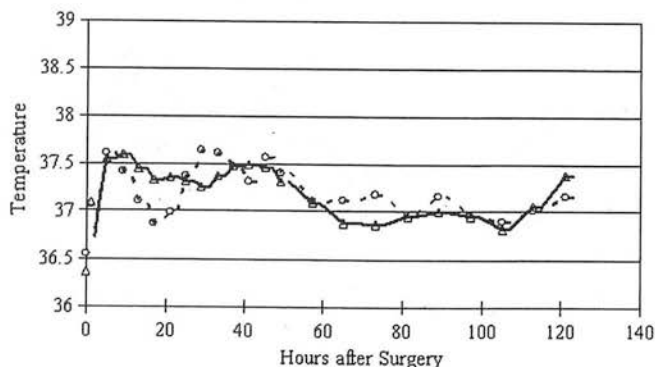
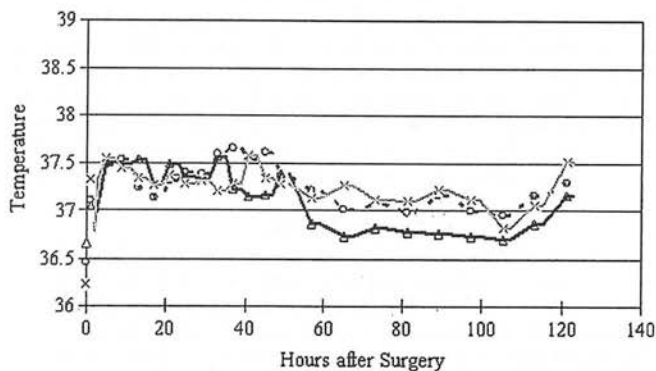
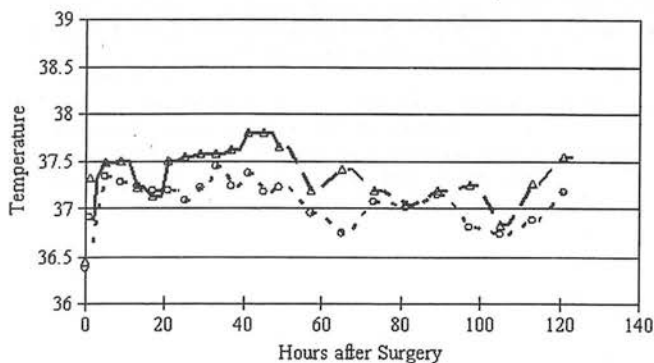
Type of Surgery	Male	Female	Total
Fronto-orbital advancement (+cranioplasty)	12	13	25
Frontofacial advancement	3	3	6
Craniectomy	6	1	7
Total	21	17	38

Pyrexia after transcranial surgery for Pfeiffer syndrome had a bimodal distribution during the first 48 hours, which is similar to the temperature course after transcranial surgery for nonsyndromic craniosynostosis (Fig. 1). This was true for both men and women (Fig. 2) and for all age-related subgroups, but pyrexia was less prolonged in those patients whose surgery was undertaken before the age of 6 months (Fig. 3). The bimodal pyrexia was higher and more prolonged in those undergoing longer surgical procedure (Fig. 4), frontofacial advancement (Fig. 5), and procedures accompanied with postoperative CSF leakage. Moreover, in cases accompanied with CSF leakage, the postoperative temperature course was more complicated than bimodal (Fig. 6).

DISCUSSION

Pyrexia has been thought to be one of the most important indicators of postoperative complication, particularly infection. Because bacterial endotoxins or exotoxins stimulate leukocytes of infected host to produce a series of pyrogens,⁷ infection can be the cause of postoperative pyrexia. However, in clinical practice, it is not unusual to encounter prolonged high temperature without any signs of complication, in daily practice of transcranial surgery. Previously, we have reported the bimodal distribution of temperature increase during the first 48 hours after transcranial surgery as a normal postoperative temperature course of nonsyndromic craniosynostosis. Similar to the previous report, we also found bimodal distribution of temperature increase during the first 48 hours after transcranial surgery for Pfeiffer syndrome. Curiously, this phenomenon was previously reported only in the cardiac surgery.⁸

Recently, Mitchell et al⁹ reported the serum cytokine secretion after cardiac surgery and concluded that tumor necrosis factor α (TNF- α) and interleukins 1 β and 8 (IL-1 β and IL-8) had bimodal secretion increase within the postoperative 48 hours, but

**FIGURE 1.** Postoperative temperature course of all over cases.**FIGURE 2.** Postoperative temperature course by sex. Male (triangular mark and solid line). Female (round mark and dotted line).**FIGURE 3.** Postoperative temperature course by age. Age younger than 6 months (triangular mark and solid line). Age older than six months and younger than 4 years (round mark and dotted line). Age older than 4 years (cross mark and gray solid line).**FIGURE 4.** Postoperative temperature course by length of surgery. Length of surgery longer than 180 minutes (triangular mark and solid line). Length of surgery shorter than 180 minutes (round mark and dotted line).

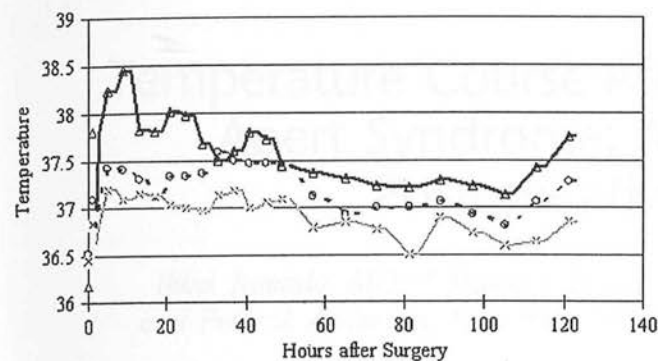


FIGURE 5. Postoperative temperature course by procedure. Frontofacial advancement (triangular mark and solid line). Fronto-orbital advancement (round mark and dotted line). Craniectomy (cross mark and gray solid line).

cytokine that demonstrated a significant association with postoperative fever was IL-6. However, the postoperative fever they defined was fever above 38°C, and we still consider that TNF- α , IL-1 β , or IL-8 may participate in the bimodal temperature increase after transcranial surgery. Because the number of articles reporting bimodal temperature increase is still limited, further investigation including measurement of the serum cytokine secretion is desirable to determine the mechanism of cytokine interaction in pyrexia after transcranial surgery.

Concerning patients' age at surgery, pyrexia was less prolonged in those patients whose surgery was undertaken before 6 months old, and this finding was similar to our previous report in which patients with simple craniosynostosis who were treated before 6 months had less pyrexia than other age groups. We speculate that this may be because flexibility of the cranial bone at this stage made surgical manipulation easier or because patients younger than 6 months had not undergone extensive surgery.

In the previous studies,^{10,11} the correlation between the length of surgery and incidence of complication has been suggested. We also have reported about the incidence of lower pyrexia in sagittal synostosis and speculated that it might be caused by the differences in the length of surgery. In the presented study, higher, prolonged temperature course was found in those undergoing longer surgical procedure, frontofacial advancement, and procedures accompanied with postoperative CSF leakage. There were no patients in this series who presented meningitis after CSF leakage. This finding of higher, prolonged pyrexia in those undergoing extensive surgery including frontofacial advancement is more likely to be complicated by CSF leakage. Cerebrospinal fluid leakage results in a more complex, swinging pattern of postoperative temperature changes than simply bimodal. We suggest that postoperative complex pyrexia may be an indicator of complication and that careful observation for the pattern of temperature increase within the first 48 postoperative hours may benefit to the early finding of the postoperative complication. However, because the number of patients investigated in the current

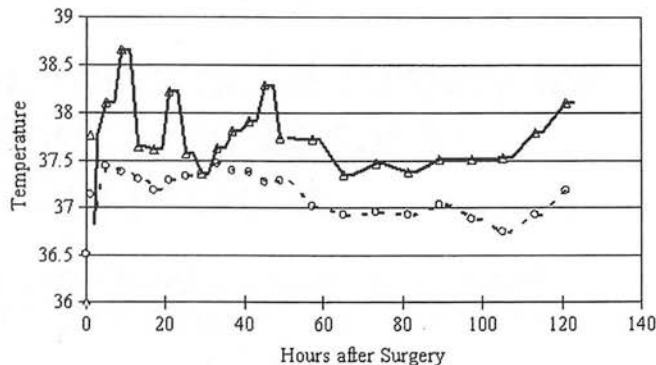


FIGURE 6. Postoperative temperature course by CSF leakage. Cerebrospinal fluid leakage (+) (triangular mark and solid line). Cerebrospinal fluid leakage (-) (round mark and dotted line).

study is limited, further investigation in a larger number of patients would clarify this.

CONCLUSIONS

It was concluded that even in a complex craniosynostosis syndrome, Pfeiffer syndrome, there is a bimodal distribution of temperature course. Those treating syndromic craniosynostosis should be aware of this normal phenomenon, and further investigation for the fever origin may be recommendable if the pattern of temperature increase within the first 48 postoperative hours is complex, apart from the simple bimodal pattern.

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Temperature Course After Transcranial Surgery for Apert Syndrome: A Possible Indicator for Postoperative Complication

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Background: Pyrexia after surgical procedure is a commonly observed phenomenon. However, it is not easy to distinguish pyrexia related to a postoperative complication from the normal temperature increase after surgery. The aims of this study were to establish a normal template for postoperative temperature course after transcranial surgery in patients with Apert syndrome and to investigate the correlation between the pattern of temperature increase and etiological factors.

Methods: Seventy-seven sequential case notes of patients with Apert syndrome were retrospectively reviewed to collect postoperative temperature courses. The mean change of temperature was plotted on a graph with trend line to compare the feature of the course in each possible factor. A separate group of those who underwent fronto-orbital advancement was independently evaluated.

Results: The temperature course had a bimodal distribution during the first 48 hours. However, those who underwent longer surgery, fronto-facial advancement, or those accompanied by complication seemed to have the more obvious third temperature peak around 50 hours postoperatively. This finding was also true in the independent fronto-orbital advancement group. Temperature courses without postoperative complication seemed to have bimodal distribution of temperature course; on the other hand, those with postoperative complication seemed to have the obvious third temperature peak around postoperative 50 hours.

Conclusions: Together with our previous study, it was concluded that pyrexia after transcranial surgery had a bimodal distribution as a normal course within the first 48 hours postoperatively. More-

over, it was suggested that the third temperature peak around 50 hours postoperatively could be an indicator for a postoperative complication.

Key Words: Craniosynostosis, Apert syndrome, postoperative complication, transcranial surgery, temperature increase

(*J Craniofac Surg* 2010;21: 50–53)

Pyrexia is a commonly observed phenomenon after any kind of surgery. Among different causes of pyrexia, infection is one of the most important because it can sometimes be life-threatening if overlooked. However, postoperative temperature increase can be encountered without any complication, so distinguishing fever resulting from a surgical complication from a raised temperature after surgery is not always easy. These findings are also true after transcranial surgery because Esparza and Hinojosa¹ have reported that the most frequent complication after transcranial surgery was postoperative hyperthermia (13.17% of the cases) followed by infection (8.10%). There still remains uncertainty about the significance of postoperative temperature observation, and some reports have concluded that pyrexia is not accurate enough as an indicator of complications; however, some reports concluded that pyrexia still has some relevance in clinical practice. In most of these reports, the definition of pyrexia was the temperature above a certain degree (in most, 38°C), and postoperative fever was judged on all-or-none basis.

Previously, we have investigated the postoperative temperature course after transcranial surgery from a different aspect, that is, the pattern of temperature increase. And we have reported the bimodal temperature increase as a normal temperature course in both nonsyndromic craniosynostosis² and Pfeiffer syndrome.³ Moreover, in the latter report that relates to syndromic craniosynostosis, we also have found the tendency of prolonged complex course in cases of longer surgery including fronto-facial advancement (FFA) and those accompanied by the postoperative complication. However, further investigation about a particular pattern of temperature course as a possible indicator for postoperative complication was not satisfactory because length of surgery, type of surgery, and postoperative complication could be influenced by each other and also because of the limited number of the cases.

The aim of this study was to investigate whether our previous findings for postoperative temperature course in other forms of syndromic craniosynostosis can be also applied to Apert syndrome. Second, we wished to investigate the correlation between the pattern of temperature increase and possible factors, that is, sex, age, procedure, length of surgery, and incidence of postoperative complications, after transcranial surgery for Apert syndrome, to verify our

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TABLE 1. Transcranial Surgeries for Apert Syndrome

	Male	Female	Total
FOA	35	39	74
Craniectomy	12	13	25
FFA	11	7	18
Total	58	59	117

hypothesis that some particular pattern of postoperative temperature course could be an indicator for postoperative complication.

METHODS

The database of the Australian Craniofacial Unit was used to identify all available cases of Apert syndrome in the unit. The records were observed to record postoperative temperature course, sex, age at operation, type of surgery, length of surgery, and postoperative complication. The mean temperatures of each 4 hours within 48 hours postoperatively and each 8 hours within further 80 hours postoperatively were evaluated. The mean change of temperature was plotted on a graph with trend line of 3-period average moving, using Excel (Microsoft, Redmond, WA) to find the feature of the course in each possible factor. Furthermore, the temperature course in fronto-orbital advancement (FOA) group was separately investigated to find the feature of temperature course by complications.

RESULTS

One hundred sequential patients of Apert syndrome were retrospectively identified. Among those patients, 77 patients (38

TABLE 2. Complications

Surgery No.	Surgery	Complication
18	FOA	Upper airway infection, diarrhea
24	FOA	CSF leak
41	FOA	Fecal <i>Salmonella</i>
46	FOA	Small surgical infection, RSV bronchitis
51	FOA	Fecal <i>Salmonella</i>
56	FOA	Fecal <i>Salmonella</i>
62	FOA	Viral upper respiratory tract infection
104	FOA	Atelectasis
105	FOA	CSF leak, frontal lobe infarction
114	FOA	Intraoperative atelectasis
40	Posterior craniectomy	Fecal <i>Salmonella</i>
48	Posterior craniectomy	Diarrhea
65	Posterior craniectomy	Urinary tract infection
2	FFA	CSF leak
39	FFA	CSF leak
70	FFA	CSF leak
81	FFA	CSF leak
95	FFA	Upper respiratory tract infection, facial cellulitis
109	FFA	CSF leak



FIGURE 1. Postoperative temperature course of all cases.

males and 39 females) had undergone at least 1 episode of surgery. One hundred seventeen transcranial operations were performed on these 77 patients. Transcranial operations consisted of 74 FOA with or without cranial reshaping, 18 FFA with or without distraction osteogenesis, and 25 craniectomies (most surgeries were for lambdoid sutures; Table 1). Length of surgery (not including anesthetic duration) could be identified in 61 operations.

Postoperatively, patients were managed according to the unit protocol, and paracetamol was routinely prescribed as an analgesic. Postoperatively, 7 operations were complicated by cerebrospinal fluid (CSF) leakage and 12 operations were complicated by other systemic problems considered to be possible factors for postoperative pyrexia (Table 2). Among the 74 FOA operations, 10 operations were accompanied by postoperative complications.

Temperature course after transcranial surgery for Apert syndrome had basically a bimodal distribution during the first 48 hours, which is similar to the previously reported temperature course after transcranial surgery for nonsyndromic craniosynostosis and Pfeiffer syndrome (Fig. 1). There was no significant difference between patients' sex. The third peak around 50 hours postoperatively, after 2 normal temperature peaks, seemed more obvious in those patients whose surgery was undertaken after 4 years (Fig. 2), who underwent longer surgical procedure (Fig. 3), FFA (Fig. 4), and procedures complicated by postoperative CSF leakage (Fig. 5).

Among FOA surgeries, there was no statistically significant difference in length of surgery between 2 groups (with or without complications). Temperature courses without postoperative complication seemed to have bimodal distribution of temperature course; on the other hand, those with postoperative complication seemed to

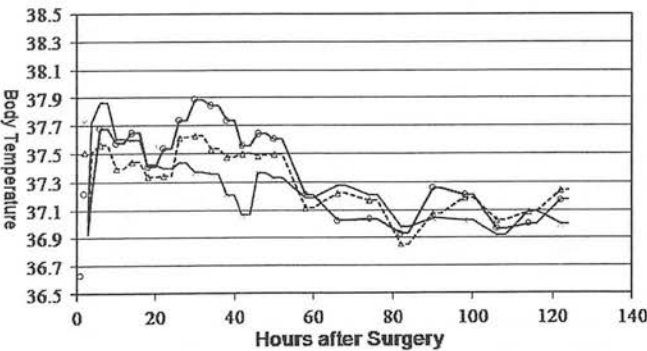


FIGURE 2. Postoperative temperature course by age. Round mark and solid line indicate age younger than 6 months; triangular mark and dotted line, age between 6 months and 4 years; cross mark and gray solid line, age older than 4 years.



FIGURE 3. Postoperative temperature course by length of surgery. Round mark and solid line indicate surgery shorter than 180 minutes; triangular mark and dotted line, surgery longer than 180 minutes.

have trimodal distribution (Fig. 6), which had the third peak around 50 hours postoperatively.

DISCUSSION

Overview of the Cases

In the current study, we could find the bimodal pattern of postoperative temperature course as a feature of whole cases (Fig. 1), similar to the previously reported temperature courses in nonsyndromic and Pfeiffer syndrome cases. Apert syndrome is known as a complex craniosynostosis sometimes accompanied by brain anomalies, including agenesis of the corpus callosum and septum pellucidum, and ventriculomegaly.^{4,5} Given the result that no significant difference was observed in the temperature course between Apert syndrome and other synostosis, it seems that the central nervous system anomaly of Apert syndrome does not influence the cascade of temperature increase via hypothalamus. Consequently, together with our previous finding in nonsyndromic craniosynostosis and Pfeiffer syndrome, we consider this bimodal temperature distribution as a common feature of temperature course after transcranial surgery.

Sex

We have already reported that there was no significant difference between patients' sex, regarding temperature course after transcranial surgery for Pfeiffer syndrome.³ This was also true in the current study; consequently, it would be concluded that difference

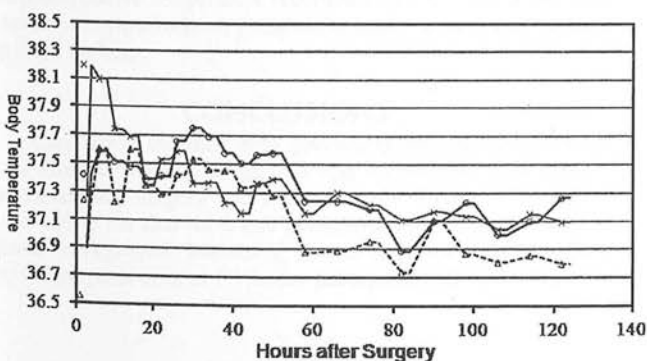


FIGURE 4. Postoperative temperature course by procedure. Round mark and solid line, FOA; triangular mark and dotted line, craniectomy; cross mark and gray solid line, FFA.

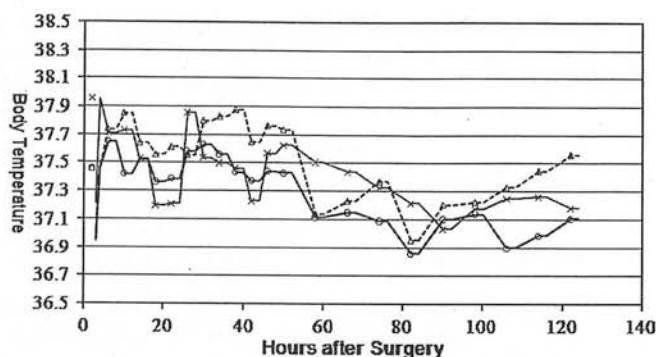


FIGURE 5. Postoperative temperature course by complication. Round mark and solid line indicate no complication; triangular mark and dotted line, complication other than CSF leakage; cross mark and gray solid line, CSF leakage.

in sex does not influence the temperature course after transcranial surgery.

Patients' Age

Regarding the patients' age at surgery, we have previously reported that pyrexia was less prolonged in those who underwent surgery before 6 months old and speculated that might be because patients younger than 6 months had not undergone extensive surgery.³ In the current study, this phenomenon was not obvious, but instead of that, we could find the more significant feature of the third peak around 50 hours postoperatively in the patients whose surgery was undertaken at 4 years old (Fig. 2). Interestingly, this unique peak was also obvious in the patients who underwent surgery longer than 180 minutes, FFA, and had postoperative complications (Figs. 3–5). This was almost the same finding as we have described in our previous study for Pfeiffer syndrome.³ However, because length of surgery, type of surgery, and postoperative complication could influence each other, we made another investigation to clarify the responsible factor for the third temperature peak.

Further Investigation in the FOA Group

It seems to be natural that older patients tend to undergo longer and more complex surgery, hence they are prone to have a postoperative complication. However, in further analysis in the FOA group made to evaluate the hypothesis that postoperative complex pyrexia may be an indicator of complication, we could identify the feature of temperature increase in this procedure was unrelated the length of surgery (Fig. 6). Consequently, we speculate that the third

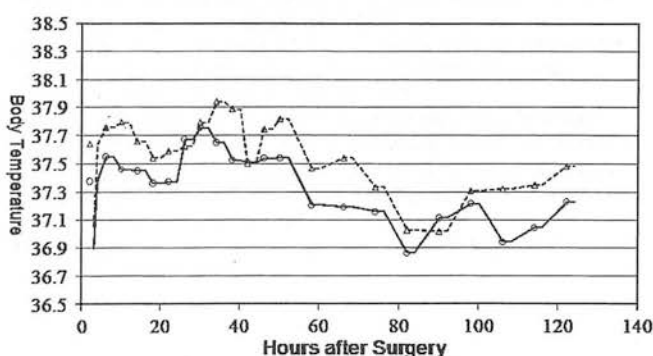


FIGURE 6. Postoperative temperature course by complication in the FOA group. Round mark and solid line indicate no complication; triangular mark and dotted line, complication (+).

temperature peak around 50 hours postoperatively could have the potential to be an indicator for postoperative complication.

Significance of Postoperative Temperature Measurement

Some arguments about the value of postoperative routine temperature observation still remain. Previously, considerable numbers of studies have already revealed inaccuracy of postoperative pyrexia as an indicator for postoperative complication.⁶⁻¹³ Also recently, Vermeulen et al¹⁴ analyzed data of 284 patients in a prospective and triple-blinded way and concluded that routine temperature measurement is of limited value in the detection of infection after elective surgery for noninfectious conditions and even advised to abandon routine postoperative temperature measurements. However, as Dellinger¹⁵ stated in his editorial commentary, prolonged or late-onset postoperative fever is still considered to have some important meaning in postoperative patient care.^{16,17} The Task Force of the Society of Critical Care Medicine and the Infectious Diseases Society of America reported the recommendation that stated, "New onset of temperature $\geq 38.3^{\circ}\text{C}$ is a reasonable trigger for a clinical assessment but not necessarily for a laboratory or radiological evaluation for the presence of infection."¹⁸ There is also the suggestion to use temperature as an indicator combined with other factors including white blood cell count, serum urea nitrogen level, and fever onset after the second postoperative day.¹⁹ However, most of those research have been based on a certain definition for pyrexia (in majority, temperature $>38^{\circ}\text{C}$) and different from our attitude toward temperature assessment. Although it is difficult to set a normal temperature course in every single type of surgical procedure, it is acceptable to use a pattern of temperature change as a baseline because normal temperature can be different for each person. Other than our previous report, bimodal distribution of temperature increase during the first 48 hours was previously reported in the cardiac surgery.²⁰ According to the report of Mitchell et al,²¹ tumor necrosis factor α , interleukin (IL) 1 β , and IL-8 had bimodal secretion increase within 48 hours postoperatively, although the cytokine that demonstrated a significant association with postoperative fever was IL-6. The interaction of each cytokines is complex,²² but there seems to be a normal pattern in secretion cascade of cytokines in temperature increase after transcranial surgery. It can be dangerous to rely too heavily on this template because infection can occur without pyrexia. We believe that using the bimodal pattern as a normal template, a third peak around 50 hours postoperatively raises the possibility of postoperative complications.

The phenomenon we have reported in this study is still a retrospective observation, and there was variance in the frequency of temperature measurement. We have now altered our protocol to have postoperative temperature recorded every 4 hours in the first 72 hours postoperatively. A prospective study is underway to confirm these findings.

CONCLUSIONS

Considered together with previously reported temperature course after transcranial surgery, it was reconfirmed that pyrexia after transcranial surgery had a bimodal distribution as a normal course within the first 48 hours postoperatively, regardless of the patients' background. Moreover, it was suggested that the third temperature peak around 50 hours postoperatively had the possi-

bility to be an indicator for postoperative complications. Although further investigation is desirable to investigate accuracy as an indicator, to be aware of these phenomenon would be useful in the management of patients who underwent transcranial surgery.

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Management of cranial deformity following ventricular shunting

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Abstract

Purpose Ventricular shunt-induced craniosynostosis is a widely recognised cause of secondary craniosynostosis. We reviewed the management and long-term outcome of the cases of cranial deformity post cerebrospinal fluid shunting in our unit and compared these with previously published series.

Methods The Australian Craniofacial Unit and Department of Neurosurgery database was searched to identify cases of ventricular shunt-induced cranial deformity and a case note review was undertaken.

Results Eight cases were identified, and all were shunted within 6 months of birth. Our patients required shunting with a low pressure valve for hydrocephalus secondary to either aqueduct stenosis or intraventricular haemorrhage. The diagnosis was made following computed tomography (CT) three-dimensional surface reconstruction of the skull. Two cases of confirmed suture fusion were treated with cranial vault remodelling and programmable shunt insertion. In six cases, the sutures were not completely fused on the CT images despite a scaphocephalic head shape. These patients were managed conservatively with close monitoring.

Conclusion Cranial vault remodelling together with insertion of programmable shunt valve is indicated in CT confirmed cases of secondary craniosynostosis.

Keywords Secondary craniosynostosis · Hydrocephalus · Ventricular shunt · Sagittal synostosis · Scaphocephaly · Programmable shunt valve

Introduction

Ventricular shunting is a well-established treatment for hydrocephalus in infancy [1]. However, it has been recognised that decompression of the cerebral ventricles may interfere with the cranial vault growth, and in some cases, this leads to premature fusion of the sutures. This was first reported by Strenger [2] in 1963, and since then, ventricular shunting has been a widely recognised cause of secondary craniosynostosis [3–7]. The published incidence of this complication varies between 1.0% and 12.4% [4, 5, 8]. Surprisingly, however, there is a marked paucity of information in the literature about this condition especially in relation to its management. We wish to review the cases of secondary synostosis from our unit to assess management and long-term outcome of this rare condition.

Method

The Australian Craniofacial Unit and Department of Neurosurgery database was searched to identify cases of ventricular shunt-induced craniosynostosis. Inclusion in the study required confirmation of scaphocephaly according to March of Dimes cranial index of scaphocephaly and ventricular shunting in the first 12 months of life. Cases of syndrome-associated scaphocephaly were excluded. A case note review was undertaken to confirm that the inclusion criteria were met and to assess the varying management and progress of these children.

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Table 1 Individual patient data

Patient	Diagnosis	Prematurity	Age at initial shunt	Shunt valve pressure	Age abnormal head shape noted	CT confirmed suture fusion	Age at cranial vault remodelling
L.E ♀	Aqueduct stenosis	No	2 days	Low	7 months	Yes	14 months
T.W ♂	Aqueduct stenosis	No	4 weeks	Low	2 months	No	N/A
D.R ♀	IVH	Yes	4 months	Low	10 months	No	N/A
J.L ♂	IVH	Yes	4 months	Low	9 months	Partial	N/A
J.H ♀	IVH	Yes	5 weeks	Low	9 months	Yes	12 months
C.T ♂	E.Coli meningitis	Yes	2 months	Low	6 months	No	N/A
B.H ♂	IVH	No	5 weeks	Low	3 months	No	N/A
D.N ♂	IVH	Yes	5 months	Low	7 months	No	N/A

All ages are not corrected for prematurity

Results

Eight cases of scaphocephaly resulting from ventricular shunting were identified, and no one was excluded. There were three girls and five boys. All were shunted within 6 months of birth (Table 1). Two cases required shunting for hydrocephalus secondary to aqueduct stenosis, five were shunted for hydrocephalus following intraventricular haemorrhage and one was shunted after meningitis. Low pressure shunt valves were inserted in all cases. The children were followed up at a minimum of three monthly in their first year of life and during this routine appointment, the abnormal head shape was noted which prompted a computed tomography (CT) three-dimensional surface reconstruction of the skull for suspicion of secondary craniosynostosis. Two cases were treated with cranial vault remodelling and programmable shunt valve insertion (Figs. 1, 2 and 3). In five cases, the radiological appearances of the sagittal suture appeared to be patent, although thickened bone was found adjacent to the sutures. These cases were managed conservatively with close follow-up and serial imaging. In one case, the sagittal suture was only

partially fused; therefore, an attempt was made to expand the cranial vault by inserting a programmable shunt valve and then progressively increasing the valve opening pressure. This was abandoned when the child started exhibiting signs of raised intracranial pressure. He has since been managed conservatively as well. One child died at the age of 7 years old following acute hydrocephalus secondary to shunt obstruction. The other seven children have varying degrees of developmental delay on formal neuropsychology assessment without clinical signs and symptoms of raised intracranial pressure. In two of the cases managed conservatively, there has been a marked improvement in the head shape without any radiological evidence of progressive suture fusion.

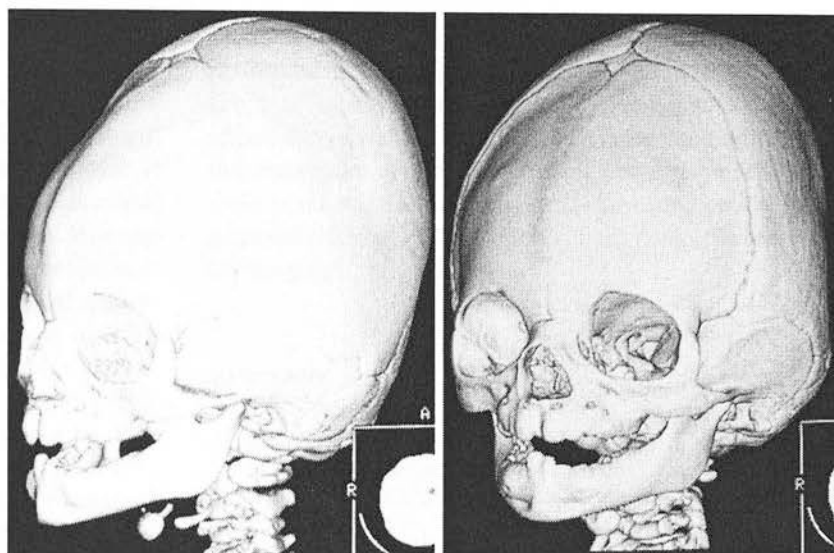
Discussion

The prevalence of congenital and infantile hydrocephalus has been estimated as 0.48 to 0.81 per 1,000 live and still births [9–11]. Hydrocephalus occurs in approximately 35% of infants with intraventricular haemorrhage and 15–20% of

Fig. 1 Abnormal head shape secondary to ventricular shunting



Fig. 2 Pre-cranial vault remodelling surgery



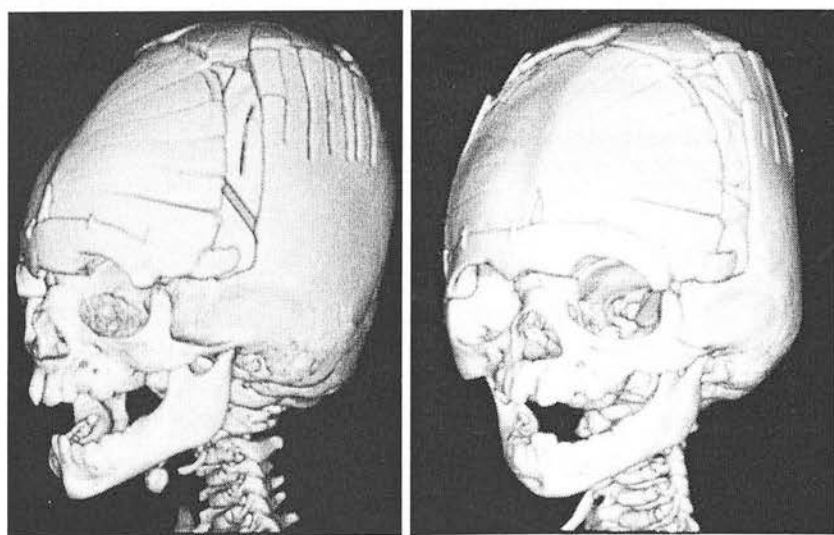
those will require cerebrospinal fluid diversion by means of ventricular shunting procedures [12]. We could find no significant series published describing the management of shunt induced craniosynostosis despite it being a well-acknowledged complication of shunting. To date, we believe that this will be the largest case series reviewing the management of shunt-induced cranial deformity.

The major complications associated with uncorrected craniosynostosis include increased intracranial pressure, altered cranial base resulting in facial asymmetry, malocclusion as well as potential adverse effect on the psychological well-being [13, 14]. There has been limited data published specifically in relation to the repair of secondary craniosynostosis. Some studies have recognised the increased complexity involved in the repair of the cranial vault abnormality in these patients and various methods have been explored to maintain the skull shape and prevent

recurrence [3, 4, 7, 8, 15]. There are, however, also those that strongly discourage surgical correction in these patients arguing that the operative risks are unacceptable in these patients who are not at risk for developing raised intracranial pressure [3, 4].

Parasagittal and linear craniectomies were described as a method to treat secondary synostosis by Roberts et al. [5] and Kloss [6]. Alternatively, Schendel et al. [7] reported good results with at a minimum sagittal strip craniectomy and biparietal osteotomies as well as the addition of an occipital and frontal remodelling in those cases with severe protrusion. The bone flaps were rigidly secured with transverse microplates to maintain the expanded shape and prevent recurrent collapse [7]. More recently, expansile springs were utilised to treat patients with scaphocephaly secondary to ventricular shunting [16]. This method was advocated as an advantage over previous craniofacial reshaping techniques in terms of

Fig. 3 Postcranial vault remodelling surgery



reducing morbidity and blood loss through limited dissection and shorter operative time.

Six patients did not have complete fusion of the cranial sutures on CT despite their scaphocephalic head shape. Davis and Lauritzen suggested in their paper published in May 2008 [16] that in such cases, craniosynostosis would have inevitably resulted and, therefore, advocated intervention before onset. We opted to manage these patients conservatively with close clinical and radiological follow-up. As reported above, two patients achieved a remarkable improvement in their head shape without any intervention; therefore, it is unnecessary to place all these patients through the risks of an operation based solely on their clinical head shape. The close monitoring is specifically directed towards early detection and prompt intervention in those patients who subsequently develop radiological evidence of fused suture.

The cranial deformity seen in our cohort was a scaphocephalic shape as are in several other reported cases. We utilised the same calvarial remodelling technique as that used in primary sagittal synostosis in our unit to achieve a near normal cranial contour. This is a safe and appropriate procedure in those with CT-proven craniosynostosis. Additionally, we believe it is imperative to use a programmable shunt valve to dilate the ventricles postoperatively to prevent the development of a subdural fluid collection which can be expected given the expanded cranial vault. It is prudent to be cautious despite published series of Shuster et al. [15] reporting that this was not an observed complication because the small cohort did not reach statistical significance and the development of a subdural collection is potentially a consequential even though as yet a theoretical one.

Our experience has led to a change in our practice. We now only insert programmable shunt valves in patients less than 12 months old, the rationale being to maintain physiological tension across the cranial sutures and conceivably prevent premature fusion by careful regulation of ventricular decompression. Alternatively, an endoscopic third ventriculostomy is conducted in appropriate cases. This restores physiological cerebrospinal fluid circulation circumventing the problems associated with ventricular over-drainage.

We conclude that cranial vault remodelling should only be performed in those cases with radiological evidence of total single or multiple cranial suture synostosis and that post remodelling insertion of a programmable shunt valve is an important part of the management strategy. Furthermore, there is no role for a prophylactic corrective procedure in patients with cranial deformity without complete fusion on CT imaging.

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Simultaneous multiple vector distraction for craniosynostosis syndromes[☆]

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KEYWORDS

Apert syndrome;
Pfeiffer syndrome;
Osteotomy;
Distraction
osteogenesis

Summary Syndromic craniosynostoses are commonly treated conditions in craniofacial units. The features of the common syndromes (Apert, Pfeiffer and Crouzon) all include craniosynostosis, mid-face hypoplasia and ocular proptosis. The craniofacial management of a child with these syndromes through to adulthood may require a number of surgical interventions to allow brain development, to provide an adequate airway, to prevent corneal ulceration and to provide a functional dental occlusion. The management of these different priorities into timed interventions in our unit is determined by established protocols.

We report two cases that underwent simultaneous mid-face (Le Fort III) and fronto-orbital osteotomies followed by distraction but using different vectors to advance the upper and mid-face regions (to achieve all treatment goals) in a 12-year-old boy and a 16-year-old girl.

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Case reports

Case 1

A 12-year-old boy with Apert syndrome was referred from an outpatient clinic in Indonesia for review. He had previously undergone a fronto-orbital advance at the age of 1 year with the procedure repeated at the age of 6 years. In

addition, he had also undergone four procedures to separate his fingers.

Currently, his major problems were related to his mid-face hypoplasia and persistent frontal flattening. This resulted in a restricted upper airway and corneal ulceration due to the limited cover provided by the position of both upper and lower eyelids. He also had a limited diet because of his abnormal dental occlusion. The forehead position also contributed to the problem of corneal exposure as it influenced the position of the upper eyelids and it was also considered to be cosmetically poor (Fig. 1(A) and (B)).

After multi-disciplinary assessment and review it was decided to address all of these problems with both a forehead and mid-face advancement.

^{*}Part of this report was presented at the 3rd International Congress on Cranial and Facial Bone Distraction Processes, Paris, France, 2001.

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Figure 1 (A) AP and right lateral photographs demonstrating the retruded forehead, hypoplastic mid-face and ocular proptosis. (B) Pre-operative lateral cephalogram, demonstrating the hypoplastic mid-face. Note the plates from the previous fronto-orbital advancement.

Because of his age it was anticipated that an inadequate amount of bone graft could be harvested from his iliac crest and ribs to use to maintain a satisfactory post-osteotomy position. Therefore, to overcome this problem, distraction was proposed.

Via a coronal approach an anterior craniotomy and fronto-orbital osteotomy was undertaken and two 30 mm mid-face distractors applied to the re-unioned frontal bone and anchored on the temple providing a distraction vector with a mainly anterior component. A Le Fort III osteotomy was undertaken and the mid-face mobilised. Two more 30 mm mid-face distractors (Leibinger) were positioned on the maxilla and malars, then anchored on the temple to

provide a vector of distraction with both inferior and anterior components. Both distractors were advanced 2 mm before closure.

Post-operative recovery was uneventful. Distraction at the rate of 1 mm per day commenced the day following surgery (Fig. 2). This was stopped for the forehead once 14 mm advancement had been reached. The mid-face advancement continued until 21 mm had been reached (Fig. 3). This was allowed to consolidate for 8 weeks prior to removal and stabilisation of the position achieved using titanium plates.

His upper airway has improved on formal testing and significantly he no longer snores at night. The current position of the eyelids affords corneal



Figure 2 Post-operative lateral cephalogram demonstrating the position of the fixators.

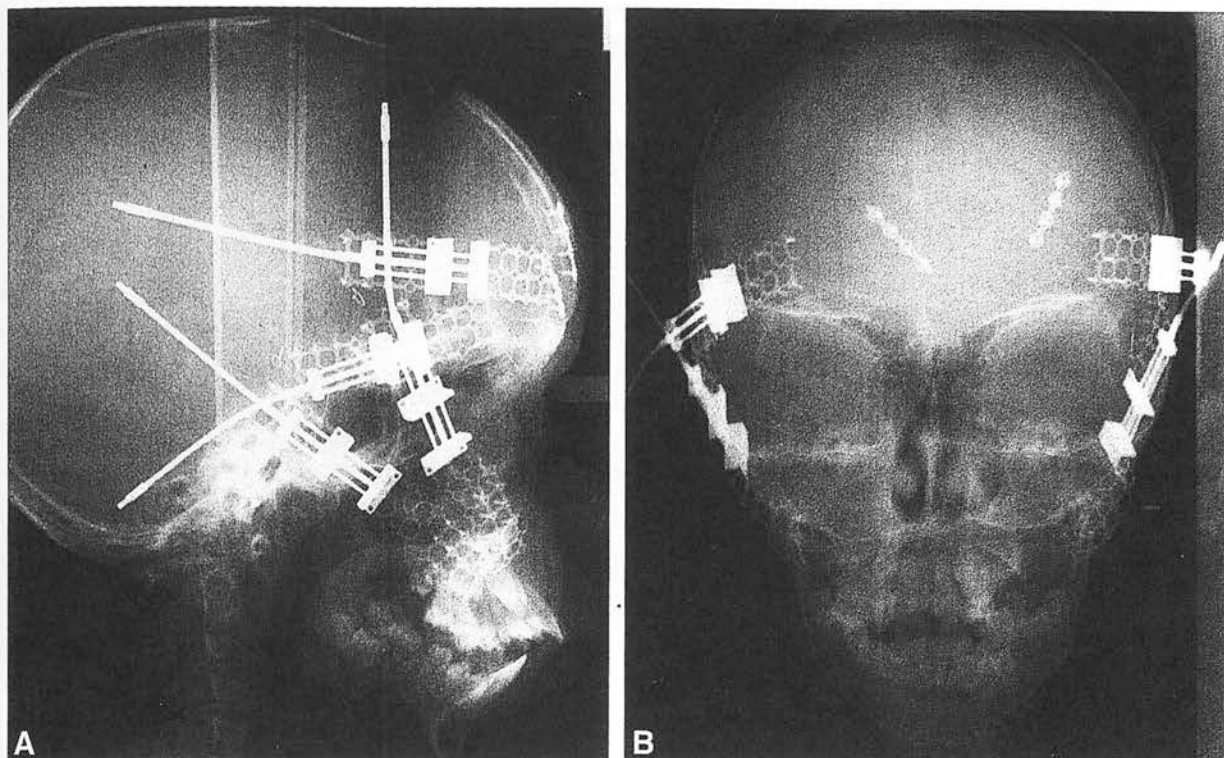


Figure 3 (A) Lateral cephalogram demonstrating the position at the end of distraction with the maxillary incisors advanced beyond the mandibular incisors. (B) AP radiograph to demonstrate the position of the distractors, (note the fixation medial to the zygomatico-maxillary junction).

protection. Additionally, he can manage an improved diet and both he and his parents are pleased with his improved appearance (Fig. 4(A) and (B)).

Case two

A 16-year-old girl with Pfeiffer syndrome was

referred from an outpatient clinic in Malaysia with corneal exposure. She was noted to marked mid-face hypoplasia with a class III dental malocclusion, ocular proptosis with corneal exposure as well as frontal flattening (Fig. 5).

After multi-disciplinary assessment and review it was decided to address all of these problems with both a fronto-orbital advancement and mid-face

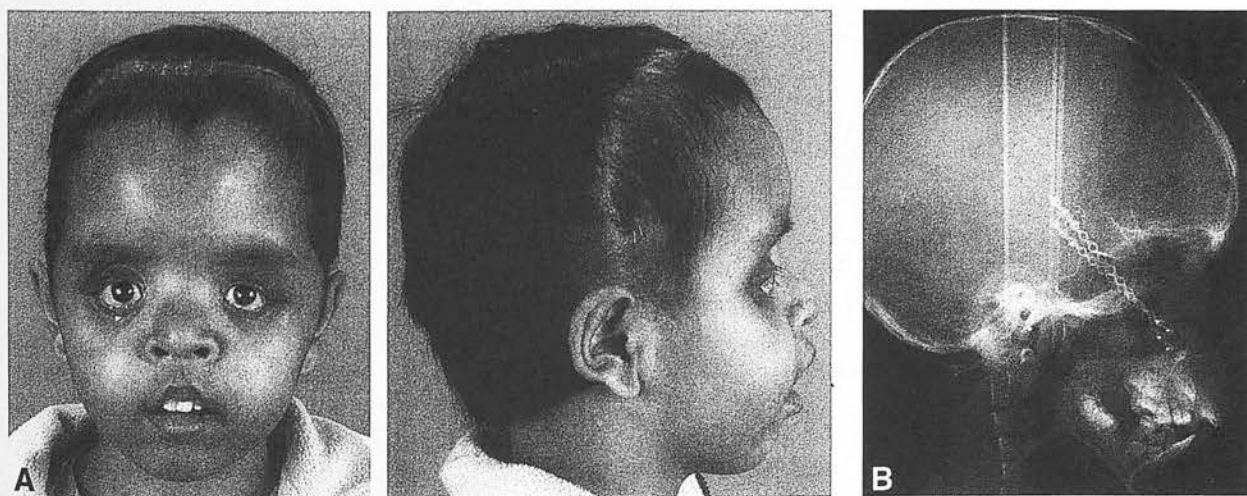


Figure 4 (A) AP and right lateral clinical photographs following removal of the distractors. (B) Lateral cephalogram demonstrating the final position. Note the bilateral cranio-maxillary fixation.

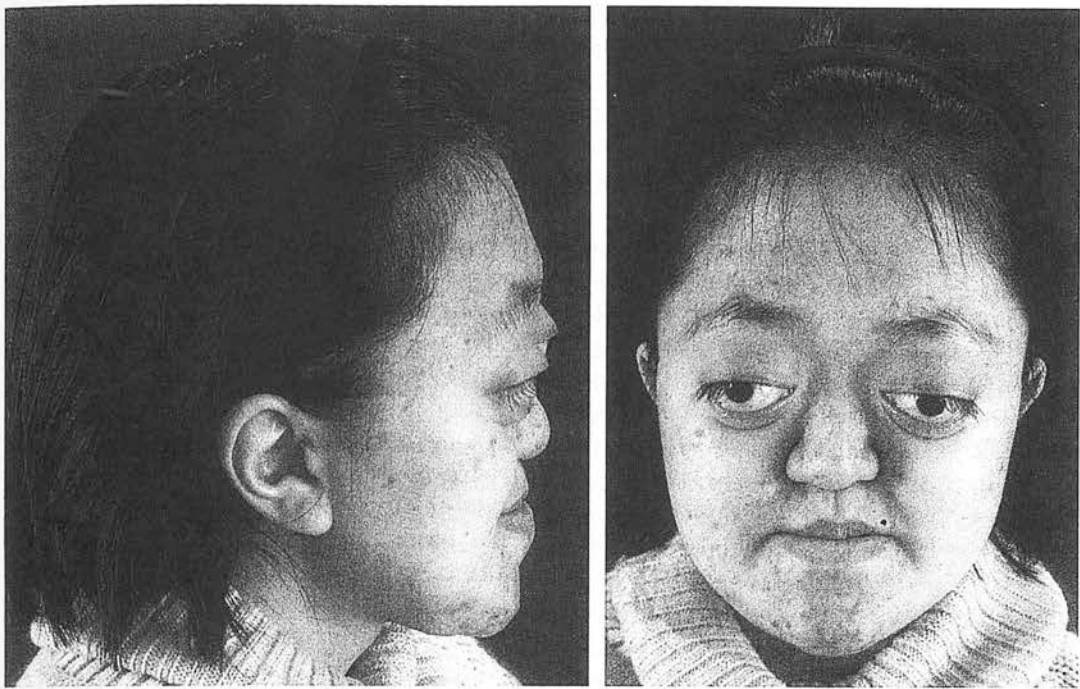


Figure 5 AP and right lateral photographs demonstrating the retruded forehead, hypoplastic mid-face and ocular proptosis.

advancement followed by distraction to achieve the large advances required in her case.
Via a coronal approach an anterior craniotomy and fronto-orbital osteotomy was undertaken and

two 30 mm mid-face distractors applied to the re-united frontal bone and orbital bar before being anchored on the temple providing a distraction vector with a mainly anterior component. A Le Fort



Figure 6 AP and right lateral photographs following removal of the distractors.

III osteotomy was undertaken and the mid-face mobilised. Two more 30 mm mid-face distractors (Leibinger) were positioned on both the maxilla and malars, then anchored on the temple to provide a vector of distraction with both inferior and anterior components. Both distractors were advanced 2 mm before closure.

Post-operative recovery was uneventful. Distraction at the rate of 1 mm per day commenced the day following. There was a minor cerebrospinal fluid leak, which settled spontaneously after 5 days, although distraction continued during this period. The distraction of the forehead was stopped once 12 mm advancement had been reached. The distractors were then removed, because one of the 'arms' had become twisted. The mid-face distraction continued until 21 mm distraction had occurred bilaterally. A period of consolidation lasting 6 weeks then followed prior to removal of the remaining distractors and placement of cranio-maxillary fixation with titanium plates. At the same operation a costochondral graft was used to reconstruct the nose. Post-operative recovery was uneventful and the early position is shown (Fig. 6). The position has proved stable and her appearance 3 years later is shown (Fig. 7).



Figure 7 AP photograph 3 years following removal of the distractors.

Discussion

Apert and Pfeiffer syndromes are characterised by craniosynostosis, mid-face hypoplasia and ocular proptosis in conjunction with limb anomalies.¹

The benefit of distraction osteogenesis as a treatment following Le Fort III osteotomy to improve the upper respiratory tract and to provide ocular protection in syndromic craniosynostosis, has been established.²⁻⁴ This includes the advantages that the stretching of the soft tissue allows a greater advancement of the osteotomy than conventional procedures.⁵ The use of distraction has already become assimilated into the treatment protocols of those with syndromic craniosynostosis requiring mid-face advancement at the Australian Craniofacial Unit. It has established a role both in skeletally immature children with mid-face hypoplasia, in their second epoch of growth⁶ and, in the skeletally mature adult.⁷

Distraction has also been extended to include its use following a more extensive facial osteotomy including the upper aspect of the face and the forehead (monobloc procedure⁸) where it has been used in children with co-existing mid-face hypoplasia.⁹ This combined upper and mid-face advancement has led to the use of multiple (four) internal distractors, placed in parallel following monobloc procedure,¹⁰ but there have been technical difficulties with this approach.⁵

In our cases, we describe the use of four internal distractors positioned so as to allow different vectors of distraction to be undertaken to the upper third and middle thirds of the face. This has resulted in improvement of the upper airway, increased ocular protection, enhanced skeletal relationship between the maxilla and mandible and an enhanced occlusion, thereby meeting all of the treatment goals in these cases.

Case 1 is an early result and there is potential for relapse from this position and the final outcome can only be measured once skeletal maturity is reached. To help prevent relapse we have used the same method of cranio-maxillary fixation, which we used in our second case⁶ in whom stability at 3 years following surgery has been satisfactory.

The distractors in these cases have been well tolerated, with the only complication being the accidental twisting of an arm in case 2 and the CSF leak also in Case two. These were both considered to be minor problems and a previous report using mid-face distractors described also reported minor problems, although these were different including weakness of the zygomatico-maxillary junction.⁵ We have been aware of this particular problem and

undertake an extended dissection to allow placement of the screws for the holding plate either side of the junction so preventing disjunction during the distraction (Fig. 3(B)).

Overall, the absence of infection in these cases we think is due in part due to the meticulous attention paid to the pin site management by both the parents and the nursing staff.

In conclusion, we report the use of distraction devices to allow different vectors of advancement between the upper and mid-face in cases of Apert and Pfeiffer syndrome following osteotomies, which is a management option in selected patients with severe syndromic craniosynostosis.

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Ophthalmic Sequelae of Crouzon Syndrome

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Purpose: To document the frequency of ophthalmic sequelae in patients with Crouzon syndrome before the influence of craniofacial surgery.

Design: Retrospective observational case series.

Participants: Seventy-one consecutive patients with a clinical diagnosis of Crouzon syndrome assessed before craniofacial or ophthalmic surgery at the Australian Craniofacial Unit between 1984 and 2000.

Methods: Review of clinical records with documentation of patient age, gender, visual acuity, refractive error, diagnosis of amblyopia, squint, eye movement dysfunction, nystagmus, fundus examination, examination of the anterior segment, interpupillary distance, and intercanthal distance.

Main Outcome Measures: The frequency of ophthalmic signs and visual impairment, defined as a visual acuity of 6/12 or less.

Results: Visual impairment in at least 1 eye occurred in 35% of patients and was bilateral in 9%. The most common cause of visual impairment was amblyopia, which was present in 21% of patients, followed by optic atrophy in 7%. Ametropia occurred in 77% of patients; 57% had hypermetropia of $\geq +2$ diopters (D) and 20% had myopia of ≥ -0.5 D. Strabismus occurred in 39% of patients. Although exposure keratopathy was observed in 15% of patients, this complication was well managed and caused no reduction in visual acuity.

Conclusions: Early detection to reduce amblyopia by correction of refractive errors, timely treatment of strabismus, and patching should be a priority for ophthalmologists and a goal of the craniofacial teams managing patients with Crouzon syndrome. Optic atrophy remains an important cause of visual impairment in these patients before decompressive craniectomy. *Ophthalmology* 2005;112:1129–1134 © 2005 by the American Academy of Ophthalmology.

Crouzon syndrome (CS) is an autosomal dominant disorder characterized by acrocephaly, exophthalmos, hypertelorism, strabismus, parrot-beaked nose, and hypoplastic maxilla (Fig 1).¹ Crouzon syndrome is the most common syndrome of more than 100 within the craniosynostosis group, yet is relatively rare, with approximately 16.5 cases per million live births.² It is usually caused by 1 of several mutations within the fibroblast growth factor receptor 2 (FGFR2) gene, which has been isolated to chromosome 10.³ This may result in premature fusion of the sutures of the cranium and base of skull.

The ocular complications of CS are numerous and include papilledema and optic atrophy from raised intracranial

pressure, corneal exposure and subluxation of the globes anterior to the eyelids secondary to exophthalmos, and strabismus. In addition, there have been sporadic reports of aniridia, anisocoria, blue sclera, cataract, corectopia, ectopia lentis, glaucoma, iris coloboma, megalocornea, microcornea, nystagmus, and optic nerve hypoplasia.⁴

Previous reports in the literature on ophthalmic manifestations have been case series or small descriptive studies.^{5,6} Larger studies have usually documented combined data from several of the craniosynostoses such as Pfeiffer's, Apert's, and Saethre-Chotzen syndromes.^{7–9} Because of the rarity of this condition, there are minimal data in the literature on the prevalence of ophthalmic sequelae in patients with CS.^{10,11} The Australian Craniofacial Unit in Adelaide, South Australia, has reviewed a large number of patients with CS referred for treatment from all over the world. In this series of 71 patients, 39 patients were referred from within Australia, 8 from Malaysia, 7 from New Zealand, 5 from Indonesia, 4 from Oman, 2 from Kuwait, and 1 each from 6 other countries. The purpose of this study was to document the prevalence of ophthalmic sequelae in this large series of CS before the influence of surgery on the visual apparatus.

Materials and Methods

A retrospective descriptive analysis of a series of consecutive patients with a diagnosis of CS was conducted. Patients with a clinical diagnosis of CS were identified from the Australian

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Figure 1. Anterior and oblique views of a 6-year-old boy with Crouzon syndrome. Note the acrocephaly, exophthalmos, strabismus, hypertelorism, parrot-beaked nose, and maxillary hypoplasia characteristic of this syndrome.

Craniofacial Unit database between the years 1984 and 2000. These patients were managed at the Women's and Children's, Royal Adelaide, and Calvary Hospitals in South Australia. Institutional human ethics committee approval was obtained for this study.

The diagnosis of CS was established after review by both the clinical geneticists and the craniofacial surgeons, based on the clinical and radiologic examination and family history. This diagnosis was based on the presence of craniosynostosis, midfacial hypoplasia, shallow orbits, and the absence of limb abnormalities. Data were obtained from ophthalmology reviews documented on a standardized ProForma, before any surgery, or the most recent assessment in those patients who had not had surgery. Exclusion criteria were (1) previous craniofacial, cranial, or strabismus surgery and (2) lack of a preoperative ophthalmology assessment.

Variables documented from the medical records included patient age, gender, visual acuity, refractive error, diagnosis of amblyopia, squint, eye movement dysfunction, nystagmus, fundus examination, examination of the anterior segment, interpupillary distance, and intercanthal distance.

Visual acuity (VA) was measured by methods appropriate to age. When determining the prevalence and etiology of impaired vision, only patients with VA corrected for refractive errors were included. Patients with VA documented as "follows fingers" were excluded from this component of the study. Patients were considered to have impaired vision if they had a VA of 6/12 or less or more than 2 Snellen lines poorer than age-adjusted normative values in at least 1 eye.¹² In the absence of a diagnosis in the case notes, amblyopia was defined as a 2-line optotype difference between the 2 eyes or from age-matched normative values in the absence of organic eye disease.¹³

Refraction was determined by cycloplegic retinoscopy and was expressed in minus cylinder form and converted to spherical equivalent for analyses. Hypermetropia was defined as a spherical equivalent of +2 diopters (D) or greater, myopia as -0.5 D or worse, and astigmatism as 0.75 D or greater difference in refractive error between the 2 principal meridians.¹² The axis of astigmatism was considered oblique if it was greater than 5° from the vertical and horizontal meridians.⁷ Anisometropia was defined as a difference in refraction between the eyes of 0.75 D spherical equivalent or greater.¹²

Statistical analysis was performed with Sigma Stat v3.0.¹⁴ Prevalence data from our study was compared with different studies using chi-square. A probability level of $P < 0.05$ was con-

sidered statistically significant. Multiple logistic regression correcting for age was used to determine whether any patient factors were associated with the presence of astigmatism.

Results

Eighty-four patients were identified from the Australian Craniofacial Unit database. Five patients were excluded because cranial, craniofacial, or squint surgery had been performed in another institution before presentation, and 8 had incomplete data. Seventy-one patients were, therefore, included in this study. The average age at ophthalmic review was 11.9 years, with a range between 4 months and 43.7 years. There were 36 males and 35 females (Fig 2).

Fifty-six patients had fully corrected VA. The 15 patients who did not have corrected VAs had a mean age of 2.2 years compared with a mean age of 15.9 years for those that did. Visual impairment ($VA \leq 6/12$) occurred in 23 eyes of 18 patients (32% of patients with documented VA). The causes of reduced visual acuity are documented in Table 1. Amblyopia resulted in visual impairment in 16 eyes of 12 patients, a prevalence of 21%. Seven of these patients had strabismic, 3 had ametropic, and 2 had anisometropic amblyopia. Four patients had no apparent amblyogenic risk factors at the time of examination. The mean age of patients with amblyopia was 18.8 years (range, 4.8–43.7 years).

Cycloplegic refractions were documented in 101 eyes of 51 patients. Table 2 presents the refractive error versus the patient age. Overall, 77% of patients had ametropia, 57% had hypermetropia of +2 D or worse, and 20% had myopia of -0.5 D or worse. To assist in comparing these results with normative data, we divided our patients into 3 equal groups, 0 to 6 years ($n = 17$), 7 to 14 years ($n = 17$), and 15 to 44 years ($n = 17$). The results are presented in Table 2. Astigmatism of 0.75 D or greater was present in 51% of patients (41% of eyes) with a mean cylindrical power of 0.9 D; 43% had with-the-rule, 43% against the-rule, and 14% oblique astigmatism, and 12% of patients had anisometropia of 1 D or greater.

Strabismus in the primary position was documented in 39% of patients. Sixteen patients had exotropia, 6 had esotropia, 2 had exotropia and hypertropia, 2 had exotropia and hypotropia, 1 had hypertropia, and 1 had hypotropia. Five of these patients had nystagmus. Sixteen (23%) patients had V-pattern strabismus,

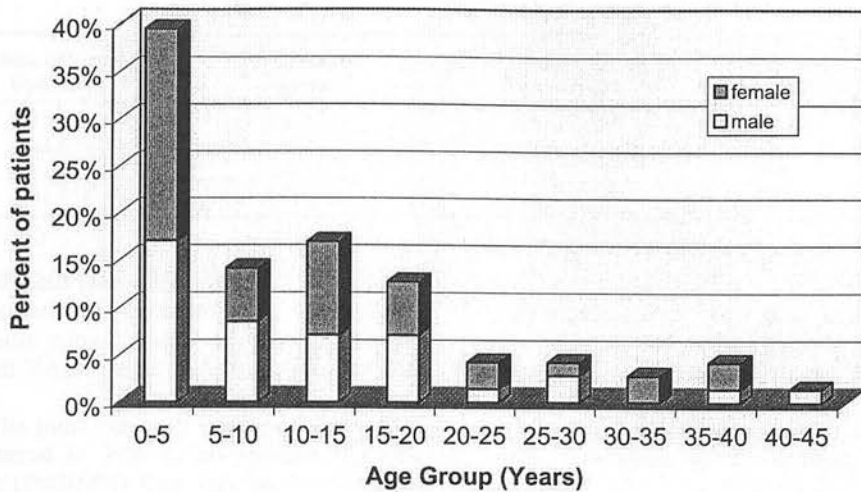


Figure 2. Distribution of patients by age and gender.

8 of which also had overaction of the inferior oblique muscle, 1 had underaction of the medial rectus, and 1 had underaction of the lateral rectus. In addition to those patients with V-pattern strabismus, a further 9 patients had ocular motility disorders ranging from grossly restricted movements to defective elevation as summarized in Table 3.

Examination of the fundus revealed optic atrophy in 9 patients (13%), papilledema in 11 (15%), retinal vessel tortuosity in 2, myelinated nerve fibers in 1, and retinitis pigmentosa (RP) in 1 patient. Papilledema was only present in patients up to 5.6 years of age, which corresponds approximately with the duration of maximal brain growth and was an indication for decompressive cranial surgery.¹⁵ Eight of the 9 patients with optic atrophy had corrected VAs documented before craniofacial surgery, and in half of these, the visual acuity was impaired in at least 1 eye. One patient had unilateral optic atrophy with a VA of 6/120, another had bilateral optic atrophy with VA documented as counting fingers in each eye, and 2 patients had bilateral optic atrophy with VA of 6/24 in 1 eye and 6/6 in the other. In each of these last 2 cases, the eye with impaired vision had a significant tropia. The patient with RP was a 21-year-old woman from India who also had optic atrophy and visual acuity reduced to 6/12 in the right eye and 6/9 in the left eye. She had a sporadic mutation of FGFR2, exon 9 with an amino acid substitution of serine 354 by cysteine (ser354cys), and no family history of RP. There has been only 1 previous publication that we are aware of in which craniosynostosis has been associated with RP.¹⁶ A case series of 3 siblings in which at least 2 had RP and all had growth deficiency, craniosynostosis, and limb changes. However, our case probably represents a chance finding of CS together with RP, because the same genetic mutation has been documented several times in the past without evidence of RP.¹⁷

Exposure keratopathy was evident in 11 patients (15%). However, no patients had visual impairment primarily as a result of this

condition, because it was managed at an early stage in all cases. Ptosis was present in 6 patients (8%).

Discussion

Crouzon syndrome is the most common syndromic craniosynostosis. It is autosomal dominant, and with improving prognosis, patients with this condition are likely to be seen more frequently in ophthalmic practice. To the best of our knowledge, this is the largest study to document the prevalence of ophthalmic sequelae in this rare condition.

Visual impairment occurred frequently in this group of patients, with 32% having VA of 6/12 or worse in at least 1 eye and 9% bilaterally. This compares with 65% and 40%, respectively, in the study by Khan et al,⁷ which combined data for several craniosynostoses, and 50% and 17% in Hertle et al's⁶ data on CS. The lower rates of impaired VA in our study probably reflect the higher mean age at presentation of our population (12 years compared with 2 years in Khan et al's study) and the assessment of afflicted relatives of referred patients. Consequently, there would be a higher proportion of patients with mild phenotypes in our sample than in these previous studies. However, because the Australian Craniofacial Unit is a tertiary referral center for overseas, some cases would have deteriorated with more severe symptoms and findings because of delay in presentation.

Structural abnormalities such as optic atrophy, exposure keratitis, cataracts, and microphthalmia have commonly been cited as causes of visual loss or impairment in CS.^{4,9,16} Our study, however, confirms the findings of Hertle et al⁶ that amblyopia was the most common cause of reduced visual acuity. We found a prevalence rate for amblyopia of 21%, which is significantly higher ($P < 0.001$) than the 2.9% in population data for children between 4 and 10 years.¹⁹ The most common structural cause of reduced visual acuity was optic atrophy. Fifty percent ($n = 4$) of patients with optic atrophy and a corrected VA documented ($n = 8$) had reduced VA. Visual fields in patients with craniosynostosis and optic atrophy are usually degraded in a concentric

Table 1. Etiology and Prevalence of Visual Impairment in Those Patients with Corrected Visual Acuities ($n = 56$)

Etiology	Prevalence
Amblyopia	21% ($n = 12$)
Optic atrophy	7% ($n = 4$)
Bilateral cataracts	2% ($n = 1$)
Retinitis pigmentosa	2% ($n = 1$)

Table 2. Refractive Error Grouped by Age

Age Group	Mean Spherical Equivalent	95% Confidence Interval	Myopia ≥ -0.5 Diopters	Hypermetropia ≥ +2 Diopters	Astigmatism ≥ 0.75 Diopters
0-6 ys	+2.2 D	1.3-3	18%	59%	59%
7-14 ys	+1.6 D	1-2.2	24%	59%	41%
15-44 ys	+1.6 D	0.8-2.4	18%	53%	53%
Overall	+1.8 D	1.3-2.3	20%	57%	51%

manner, so VA is affected late in the process.⁹ It is, therefore, possible that the rate of amblyopia quoted in our study is understated, because some patients with optic atrophy may have had normal VA if not for their strabismus and/or refractive error.

Strabismus was the most common condition leading to amblyopia and occurred in 39% of all patients. This is significantly greater ($P < 0.001$) than that seen in normal populations; in particular, a recent longitudinal study on 3126 children between 4 and 10 years found 2.7% had manifest strabismus.¹⁹ The high rate of exotropia in CS results from the obtuse angle of the orbits and the fact that many patients have altered extraocular muscle form or number.²⁰ The mean age of patients with strabismus in our series was 14 years, so any future strabismus surgery would be predominantly cosmetic in nature. Strabismus surgery is often delayed in patients with craniosynostosis syndromes, because future surgery to the orbital area may alter the degree and type of ocular deviation.²¹ However, Diamond et al²⁰ have demonstrated that in only 10 of 140 major craniofacial reconstructive operations was there a shift in primary position alignment. Greaves et al⁵ found that in 19 cases of oxycephaly and CS, no patients had a change in alignment after the Tessier procedure. Interestingly, Morax²² found that in 11 cases of craniofacial stenoses

(9 CS, 2 Apert's syndrome) examined before and after sagittal expansion of the orbits, exotropia ($n = 10$) was always reduced with orthophoria in the primary position. Vertical deviation was sometimes diminished but never disappeared entirely. On the other hand, surgery for hypertelorism often resulted in esotropia.

It has been proposed that strabismus surgery in craniosynostosis should be performed in the first 2 years, and arguably 6 months, to enable the development of binocular vision.²⁰ If craniofacial surgery involving the orbits is to be performed during this early stage, strabismus surgery should be delayed for at least 6 months afterward and preferably greater than 1 year.^{18,20,22} Given the high rates of amblyopia and strabismus seen in our study, such a regimen would be worth considering, particularly in those patients with minimal hypertelorism and young age. Earlier strabismus surgery in these cases may improve binocular vision and reduce amblyopia. However, this must be carefully considered against the likelihood of later midface surgery affecting the orbits once skeletal maturity has been reached and the difficulties obtaining perfect alignment in these complicated cases. Clinical trials comparing early with late strabismus surgery are needed to guide treatment protocols in the management of strabismus in patients with craniosynostosis.

Given the large variation in age and ethnic background in

Table 3. Examination Findings of Those Patients with Ocular Motility Disorders Other Than V-Pattern Strabismus

Patient Identification Number	Age (yrs)	Corrected Visual Acuity	Refraction	Tropia	Ocular Motility	Fundus Findings
10	12.2	RE 6/6 LE 6/9	+1.75/+0.75×100° +0.75/+1.5×85°	Intermittent XT	SR- LE	
15	0.3		+2.0 sph +1.5 sph	XT	Grossly restricted OU	Bilateral papilloedema
17	26	RE 6/6 LE 6/5	+6.5/-0.5×180° +4.5 sph	XT	MR-, LR- RE	
23	28.1	RE 6/6 LE 6/6			LR-, MR+ OU	Convulsed retinal veins
28	4.8	RE 6/12 LE 6/9	-2.0/+2.0×160° -2.0/+2.0×30°	XT	LR-, SR- RE	
39	11.2	OU CF	+4.0 sph +4.0 sph	Alternating XT	SR- OU	Severe optic atrophy
43	17.6	RE 6/6	+6.50/-3.0×20°		MR-, LR-, SR- OU	
46	10.2	LE 6/6 RE 6/9 LE 6/9	+6.50/-3.0×160° +2.5 sph OU	Variable XT	MR+ OU	Right disc blurred nasally
51	15.5	RE 6/24 LE 6/6	+5.5 sph +5.0 sph	HT RE	SR- RE	Bilateral optic atrophy

+ = overaction; - = underaction; CF = counting fingers; HT = hypotropia; LE = left eye; LR = lateral rectus; MR = medial rectus; OU = both eyes; RE = right eye; sph = sphere; SR = superior rectus; XT = exotropia.

our series of patients, we are cautious in comparing the refractive data with normal populations. It is well documented that from early childhood into adulthood there is a progression in refractive state from hypermetropia to myopia.²³ In addition, several recent and large epidemiologic studies on the refraction of school-aged children in India, Nepal, China, Chile, South Africa, and the United States demonstrates great variation in mean refraction between different ethnic backgrounds.^{24–29} Despite this, our data demonstrate a substantially greater prevalence of refractive errors compared with these normal population studies. Ametropia was present in 77% of our patients overall. Most significant is the high rate of hypermetropia and astigmatism. The rate of hypermetropia in our 7- to 14-year age group was 59%. In comparison, the highest rate of hypermetropia documented in these recent epidemiologic studies was significantly less at 19.3% ($P < 0.001$) in a white, American population aged 5 to 17 years with hypermetropia of $\geq +1.25$ D.²⁹

High rates of hypermetropia have been demonstrated in patients with craniosynostosis, although none as high as our results in large series of CS. Bertelson⁹ documented a rate of 21% for hypermetropia greater than +1.5 D in 116 patients with oxycephaly, including 12 patients with CS. Hertle et al⁶ have documented individual refractions in 25 patients with CS, and from this data it is apparent that 24% had hypermetropia ≥ 2 D. Refraction data from a recent study on 141 patients with different craniosynostoses including CS demonstrated that 42.5% of eyes had hypermetropia.⁷ The likely cause of the predominance of hypermetropia is that the shallow orbits seen in this condition result in a reduced axial length. This has not been proven, nor does it explain the relatively high rates of myopia.

The large percentage of patients with astigmatism of 0.75 D or greater (51%) compares with values of 2.8% to 19% in unaffected populations.^{24–28,30} Previous studies have documented rates of astigmatism between 72% and 43% in patients with CS. The etiology for the high rate of astigmatism is not known but has been attributed to ptosis, corneal exposure, or unequal pressure from the bony orbit. We could find no significant association between the presence of astigmatism and exposure keratopathy, interpupillary distance, intercanthal distance, strabismus, apparent exophthalmos, or optic disc pathology when correcting for age using multiple logistic regression. However, oblique astigmatism was associated with the presence of corneal exposure (odds ratio, 6.3; 95% confidence interval, 1.2–34.6.). FGFR2 gene expression has been demonstrated in the embryonic rat lens and cornea of the developing chick.³¹ Therefore, mutations of this gene could conceivably effect growth of the globe and refractive state.³²

There are some important limitations of this study. First, patients were diagnosed on the basis of phenotypic findings and did not have genotypic confirmation at the time of review. Studies have demonstrated that between 9% and 35% of clinically diagnosed patients with CS do not have genetic confirmation.^{33–35} However, the molecular genetics of the syndromic craniosynostoses are not fully understood, and these conditions display substantial genetic heterogeneity.³³ Patients with Crouzon and Pfeiffer phenotypes have

been found to have exactly the same genetic mutation. In other cases, patients with obvious CS phenotype have had no genetic mutation identified at all. We believe that phenotypic studies such as this continue to provide useful prevalence data, especially for ophthalmologists who often review patients with craniosynostosis before genetic confirmation is available. Second, many patients were assessed in infancy, before craniofacial surgery, and could have had further ophthalmic sequelae develop if not for these interventions. The risk of ophthalmic sequelae from CS if not operated on is, therefore, greater than we have documented, and this risk would vary depending on the severity of the phenotype. Finally, the retrospective nature of this study and the fact that the sample was obtained from a tertiary hospital results in an ascertainment bias favoring more severe cases. The accuracy of these results could be improved by a prospective study that follows all severities of phenotype at specific time intervals. These data do, however, enable us to identify the most common ophthalmic sequelae and direct our attentions appropriately so as to improve visual outcomes in these patients.

In conclusion, data from this study have documented the prevalence of ophthalmic sequelae in CS before surgical intervention. Visual impairment occurred in 32% of patients, and amblyopia was the leading cause of visual loss. To reduce the high rate of visual impairment, particular attention needs to be paid to correcting refractive errors, timely management of strabismus, and treating amblyopia. Optic atrophy was present in 13% of patients and continues to be a cause of severely reduced visual acuity before decompressive craniectomy. Further studies should be undertaken to determine whether regular monitoring of visually evoked potentials and perimetry can identify those at risk of optic atrophy before funduscopically visible changes. Studies on the expression of FGFR2 and related proteins in human ocular tissue and the effect of gene mutations on growth of the eye in animal models are needed and may enhance our understanding of the pathogenesis of refractive errors.

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Ophthalmic Findings in Apert's Syndrome after Craniofacial Surgery

Twenty-nine Years' Experience

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Purpose: To survey the spectrum of ophthalmic morbidity in Apert's syndrome after craniofacial surgery.

Design: A retrospective study of patients with Apert's syndrome managed at the Australian Craniofacial Unit from 1975 to 2004.

Participants: Sixty-one patients (31 females and 30 males) had final ophthalmic reviews at a mean age of 9.3 years (standard deviation, 9.2; range, 0.2–48.3; median, 8.2 years).

Methods: Patients were identified from the unit database, and case notes were reviewed. Cases that had ≤ 2 recorded variables were excluded. Demographic details, age at last ophthalmic review, and total craniofacial operations performed were documented.

Main Outcome Measures: Best-corrected visual acuity, cycloplegic refractions, strabismus, amblyopia, corneal abnormality, fundoscopic findings, and visually evoked potentials.

Results: The average number of craniofacial operations performed was 2 (range, 1–4; median, 2). Visual impairment was found in 54% of patients in at least one eye and in 19% of patients in their better eye. The most common cause was amblyopia, with a prevalence of 35%. Optic atrophy caused visual impairment in 5% of patients and corneal scarring in 8%. Sixty-three percent of patients had strabismus with more esotropia than exotropia. Ametropia was found in 69% of patients (42% were hypermetropic and 27% were myopic). Anisometropia of ≥ 0.75 diopters was present in 16 cases (50%).

Conclusions: Visual impairment is a common finding in Apert's syndrome and amblyopia is the major cause. Ametropia, astigmatism, anisometropia, and strabismus frequently occur in patients with Apert's syndrome at final ophthalmic review. Although optic atrophy was the major cause of visual loss in the era prior to craniofacial surgery, the prevalence of optic atrophy is low since the adoption of current surgical protocols. Corneal damage also contributed toward visual impairment. Early detection and adequate management of amblyopia, timely decompressive surgery before the presence of optic atrophy, and protection of the cornea should be the management goals of ophthalmologists in craniofacial units managing these patients. *Ophthalmology* 2006;113:347–352 © 2006 by the American Academy of Ophthalmology.

Apert's syndrome is a severe autosomal dominant disorder characterized by craniosynostosis, midface hypoplasia, and

syndactyly of the hands and feet.^{1,2} It is one of the most common craniofacial synostosis syndromes, yet it is a rare condition with an estimated prevalence of 12.5 to 15.5 cases per million live births.^{3,4}

Most reports on past ocular findings are either small series or case reports, or large combined studies of various craniosynostoses syndromes.^{5–10} There are little data on the prevalence of ophthalmic manifestations in Apert's syndrome. The Australian Craniofacial Unit has reviewed a large series of patients with Apert's syndrome from within Australia, South East Asia, and Central Asia during the last 29 years. The aim of this study is to determine the spectrum of ophthalmic sequelae in Apert's syndrome after craniofacial surgery.

Patients and Methods

Eighty-seven patients with Apert's syndrome who were treated between 1975 and 2004 were identified from the Australian

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Craniofacial Unit database. The records of all Apert's syndrome patients were obtained from the Women's and Children's Hospital, Royal Adelaide Hospital, Harley Eye Clinic, and the Australian Craniofacial Unit case notes. Patients who had ≤ 2 recorded ophthalmic examination variables and those who did not have any craniofacial surgery were excluded. Institutional human ethics committee approval was obtained for this study (approval no.: REC1527/11/2006).

Of the 87 patients identified from the database, 77 case notes were available for review. Seven patients with insufficient data and 9 patients who did not have any craniofacial surgery were excluded. In total, 61 patients had a confirmed diagnosis of Apert's syndrome based on clinical findings that were reviewed.

Patient profile data consisted of demographic details, such as age at last ophthalmic review, gender, country of origin, and number of craniofacial operations performed prior to the final ophthalmic review. The following ophthalmic parameters were investigated: best-corrected visual acuity (VA), cycloplegic refractions, diagnosis of amblyopia, strabismus, ocular movement abnormality, anterior segment findings, fundoscopic findings, visually evoked potentials, and other notable findings.

Best-corrected visual acuity was measured by methods appropriate to age and verbal capacity. Kay pictures, the Sheridan Gardiner test, and the Snellen chart were used and recorded as Snellen or Snellen equivalents. In preverbal children, the fixation pattern was determined as central, steady, or maintained. Visual impairment is defined as having a VA of worse than 6/12.¹¹ When determining causes of visual impairment, patients with fix-and-follow vision were excluded from this part of the study.

Refraction was obtained by cycloplegic retinoscopy; it was expressed in minus cylinder form and was converted to spherical equivalent for analyses. Hypermetropia was defined as $\geq +2$ diopters (D), myopia as -0.5 D or worse, and emmetropia as between -0.5 D and $+2$ D. Astigmatism was defined as ≥ 0.75 D between 2 meridians with axis $0 \pm 5^\circ$ as with-the-rule, and axis $90 \pm 5^\circ$ as against-the-rule, and all other axes in between as oblique. We defined anisometropia as a difference in refraction between the eyes of ≥ 0.75 D spherical equivalent. In the absence of a diagnosis in the case notes, amblyopia was defined as a 2-line optotype difference between the two eyes with no attributable or refractive cause.¹²

Results

There were 61 patients (31 females and 30 males) with Apert's syndrome who underwent at least one craniofacial surgery at a mean age of 9.3 years (standard deviation, 9.2; range, 0.2–48.3; median, 8.2 years) at last ophthalmic review. In this series, 23 patients were referred from within Australia, 18 from Malaysia, 9 from Indonesia, 3 from Singapore, 3 from Hong Kong, 2 from Thailand, 2 from Oman, and 1 from New Zealand. The average number of craniofacial surgical interventions performed was 2 (range, 1–4; median, 2). Not all sets of data were present for all parts of the study. Visual acuity was documented in 52 patients. Thirty-two patients had cycloplegic refraction data, 49 patients had data on strabismus, 48 patients had data on anterior segment examination, 51 patients had data on fundus finding, and 33 patients had data on visually evoked potentials.

Corrected VA was documented in 37 patients (74 eyes). There were also 15 patients with fix-and-follow vision and 9 without VA data. Visual impairment (VA $< 6/12$) was found in 36% of eyes (24 of 74). Furthermore, 19% of patients (7 of 37) had VA worse than 6/12 in their better eye, whereas 54% of patients (20 of 37) had VA worse than 6/12 in at least one eye. The causes of visual impairment are summarized in Table 1. Amblyopia caused visual im-

Table 1. Causes for Visual Impairment in Apert's Syndrome (N = 37)

Causes of Visual Impairment (Visual Acuity $< 6/12$)	Prevalence
Amblyopia	35% (n = 13)
Optic atrophy	5% (n = 2)
Corneal scar	8% (n = 3)
Causes undetermined due to insufficient data	n = 2

pairment in 15 eyes of 13 patients. There were 7 patients with strabismic amblyopia, 4 patients with mixed amblyopia, and 2 patients with ametropic amblyopia. Optic atrophy resulted in visual impairment in 3 eyes of 2 patients, and corneal scarring resulted in visual impairment in 4 eyes of 3 patients. However, 1 patient had significant corneal scarring caused by traumatic injury unrelated to the syndrome.

Ametropia was found in 69% of patients. Overall, 42% of eyes (27 of 64) were hypermetropic, 27% of eyes (17 of 64) were myopic, and 31% of eyes (20 of 64) were emmetropic. Astigmatism of ≥ 0.75 D was present in 35 eyes (55%), where 15 eyes (43%) had with the rule, 8 eyes (23%) had against the rule, and 12 eyes (34%) had oblique axis. Anisometropia of ≥ 0.75 D was present in 16 of 32 cases (50%).

Sixty-three percent of patients (36 of 49) examined had strabismus in the primary position. There were 16 patients who had esotropia, 11 who had exotropia, 2 who had vertical strabismus, and 2 who had unspecified manifest strabismus at primary gaze. Eleven patients (28%) had V-pattern ocular motility, and another 11 patients also had overaction of inferior oblique muscles. Five patients were noted to have latent nystagmus.

Fundus examination revealed optic disc pallor in 16% of patients (8 of 51; 6 bilateral and 2 unilateral), and papilledema in 1 patient. The visual acuities of the 2 patients with unilateral optic atrophy were poor at 6/60 and 6/36. In bilateral optic disc pallor, VA ranged from 6/6 to 6/60. Abnormal visually evoked potential was recorded in 14 eyes of 9 patients. Five of these patients had bilateral abnormalities. Of the 14 eyes with abnormal visually evoked potential, 2 eyes had pale optic discs, 9 had normal fundi, and 3 did not have any documented findings of the fundus.

Corneal abnormalities were detected in 24% of eyes (23 of 96), and they included 5 corneal scars, 4 corneal keratopathies, and 2 corneal ulcers. Corneal scars were attributed to exposure in 2 patients, with craniofacial surgery in 1 patient who also had a corneal ulcer in the contralateral eye and an unrelated accidental corneal trauma. One corneal ulcer developed after fronto-orbital advancement with release of tarsorrhaphy, and another ulcer developed in a patient with decreased corneal sensation and trichiasis.

Other ophthalmic findings included 8 patients who had trichiasis, 3 who had entropion, 3 who had ectropion, 4 who had epiblepharon, 4 who had nasolacrimal obstructions, 13 who had nonobstructive epiphora, 1 who had cataract, and 1 who had iris coloboma.

Twelve patients older than 14 years of age had completed all craniofacial surgery. Their final ophthalmic findings are summarized in Table 2.

Discussion

Among the numerous craniosynostosis syndromes described, Apert's syndrome is one of the most well known. In

Table 2. Final Visual Outcomes of Patients in the Third Phase of Growth, Older than 10 Years of Age (N = 12)

Patient No.	Age (yrs)	Visual Acuity	Refraction	Tropia in Position	Diagnosis of Amblyopia	Ocular Motility	Fundus Findings
1	15.2	RE 6/12 LE 6/24	4.25/-1×110 3/-1×70	RE nil LE esotropia	RE no LE yes		Normal
2	48.3	RE 6/9	1.25/-0.75×55				Bilateral disc pallor
3	14.6	LE 6/6 RE 6/9 LE HM	0.25/-0.75×155 3.5 spherical 2.5 spherical	RE nil LE esotropia	RE no LE yes	IO+ IO+	Normal
4	21.6	OU 6/60	-3/-2.5×90	OU nil	RE no		Bilateral disc pallor
5	16.4	RE 6/6 LE 6/12	-0.75/-2.75×0	OU nil	LE no RE no	IO+, upgaze restriction IO+, upgaze restriction	Normal
6	17.9	RE 6/6 LE 6/12			RE no LE yes		Normal
7	17.9	OU 6/6		RE nil LE hypertropia			Normal
8	17.4	RE 6/9 LE 6/18		RE nil LE esotropia	RE no LE yes		Normal
9	17.2	OU 6/12	1/-4×135	RE esotropia			Bilateral disc pallor
10	14.5	RE 6/18 LE 6/9	-1.75/-4×45 4/-1.25×119	LE nil RE esotropia	RE yes LE no	V exotropia V exotropia	Normal
11	16.8	OU 6/9	2.5/-0.75×148	LE nil	RE no LE no	IO+ IO+	Normal
12	44.3	RE 6/18 LE 6/24		RE exotropia LE exotropia	RE yes LE yes	IO+ IO+	Normal

HM = hand movements; IO+ = inferior oblique overaction; LE = left eye; OU = both eyes; RE = right eye; V = V pattern.

addition to craniosynostosis and midface hypoplasia, syndactyly of the hands and feet is required to establish the diagnosis of Apert's syndrome. Fusion of the second, third, and fourth digits is the most common type of syndactyly in Apert's syndrome. Fusion of the second to fifth digit with a free thumb is the second most common type.^{1,2,13}

The typical features of Apert's syndrome include hyperacrobrycephaly, low-set ears, ocular hypertelorism, variable degree of proptosis, downslanting palpebral fissures, midface hypoplasia, and a prominent parrot-beak nose (Fig 1).^{1,2,13} Apert's syndrome (along with Crouzon, Pfeiffer, and Beare-Stevenson syndromes) is associated with allelic mutations of the fibroblast growth factor receptor 2 (FGFR2) gene, but unlike the other syndromes, the mutational spectrum of Apert's syndrome is remarkably specific.¹⁴⁻¹⁶ Analyses of unrelated patients with new mutations all have 2 specific C to G transversions in the FGFR2 gene producing a Ser252Trp and Pro253Arg substitution.^{15,16} Studies also suggest that advanced paternal age is a risk factor for new mutation in Apert's syndrome.^{15,17}

The ocular problems in Apert's syndrome result from disproportionate growth of the brain and the eye. Premature closure of multiple cranial sutures results in the restriction of intracranial and orbital space expansion.^{1,2,13} The orbits become lateralized and shallow and are no longer able to adequately protect the globe, which results in proptosis, strabismus, and hypertelorism.^{18,19} Visual loss is the most severe ocular manifestation and can result from exposure

keratitis and corneal scars, amblyopia, and optic atrophy.^{18,20,21} Other ophthalmic findings described include lacrimal apparatus dysfunction, structural alteration, absence of extraocular muscles, iris coloboma, ocular albinism, keratoconus, ectopia lentis, congenital glaucoma, staphyloma, cataract, and medullated nerve fibers.^{6,9,10,20-23} An adequate ocular evaluation before and after craniofacial intervention is very important. Ophthalmic concerns of progressive proptosis with risk of severe globe exposure, papilledema, and progressive visual failure from optic nerve compression in the neonatal period or infancy necessitate an immediate plan of craniofacial surgery.¹⁹ To the best of our knowledge, there are no studies that investigate the prevalence of ophthalmic sequelae in patients with Apert's Syndrome after craniofacial surgery.

The surgical management protocol at the Australian Craniofacial Unit has been based on the 3 epochs of growth.²⁴ In the early period up to 12 months, the surgical approach aims to expand the cranial base and protect the superior orbit with fronto-orbital advancement, and to decompress the brain with posterior and lateral craniectomies. In the intermediate period from 1 to 10 years old, orbitostenosis from progressive maxillary hypoplasia is managed with midfacial advancement to protect the globe at the inferior margin, and to relieve concomitant upper airway restriction. In severe cases of craniosynostosis, cranial space expansion is achieved by generous fronto-orbital advancement or large bilateral decompressive craniotomies, or a



Figure 1. Clinical features of a patient with Apert's syndrome. Note turriccephaly, hypertelorism, antimonogloid slant, proptosis, low-set ears, midface hypoplasia, parrot-beak nose, and open bite.

combination of both. In the late period, from year 10 onwards, the focus is toward definitive facial surgery for a good aesthetic result.

In this study we found that visual impairment is common, and it occurred in 54% of patients in at least one eye. This compares with 73% in 15 patients with Apert's syndrome,⁵ 32% in 56 patients with Crouzon syndrome,¹² and 65% in 141 cases of a combined population of craniosynostosis syndromes.⁸ In population studies, the prevalence of visual impairment equal to or worse than 6/12 in school-age children is considerably lower, ranging from 1.8% to 7.4%.^{25,26} Although structural abnormalities are cited to be the major cause of visual loss in craniosynostosis,^{7,27-29} our study suggests that amblyopia is the major cause of visual impairment in Apert's syndrome. The prevalence of amblyopia is 35%, compared with only 3.9% to 6.5% in population studies of school-age children.^{25,26,30} The focus of ophthalmic review in patients with Apert's syndrome should be the early detection of amblyopia and the associated amblyogenic risk factors and timely occlusive therapy. All causes of amblyopia need to be fully evaluated at the initial presentation. Early strabismus surgery before the completion of craniofacial surgery has been advocated if amblyopia and loss of fusion significantly impair vision.^{31,32} However, delayed strabismus surgery has also been advocated, because in children with complex craniofacial malformation, strabismus results from interplay of multiple factors including outward and inferior rotation of bony orbits with orbital and ocular extorsion, and in some cases absence and microscopic anomalies of extraocular muscles which may not be corrected with conventional strabismus surgery alone.^{9,13,22,23,33} The timing of strabismus surgery needs to be considered in conjunction with the priority of craniofacial surgery for functional problems,

effectiveness of other amblyopia therapy, age, and psychological problems associated with strabismus.

Strabismus is a frequent finding in Apert's syndrome, with a prevalence of 63%. This study found a higher prevalence of esotropia than exotropia, which is consistent with the finding by Khan et al⁸ at final ophthalmic review. Craniofacial surgery does not normally result in a change of ocular alignment.³¹ The phenomenon of more esotropia than exotropia after surgery may be due to a change of the pattern of strabismus from V exotropia to A-pattern esotropia observed immediately after surgery.³⁴ The majority can be expected to revert spontaneously back to the original pattern within 6 to 12 months; therefore any surgical correction of postoperative strabismus should be delayed for at least 6 months to a year after periorbital stripping in craniofacial surgery.

The study shows a high prevalence of refractive errors (69%), especially hypermetropia (42%) in patients with Apert's syndrome, which mirrors the refractive findings in Crouzon syndrome (77% of ametropia and 57% of hypermetropia).¹² Direct comparison between prevalence of refractive errors in population studies and in patients with Apert's syndrome may not be valid due to the large differences between age, sex, and ethnicity.³⁵ However, the highest prevalence of ametropia (23%) and hypermetropia (27%) across different population studies ranging from 1 to 15 years old are still lower than this study.^{25,26,35-38} It has been shown that hypermetropia of ≥ 2 D is significantly associated with strabismus and amblyopia.^{39,40} Anisometropia (50%) and oblique astigmatism (34%) are also common findings in Apert's syndrome. The presence of oblique astigmatism during childhood is strongly associated with amblyopia,⁴¹ and both anisometropia and oblique astigma-

tism correlate with the presence of amblyopia in an adult population.⁴²

Using the current surgical management approach, the prevalence of optic atrophy in this study is much lower compared with the era before a multidisciplinary approach to craniofacial surgery. Before craniofacial surgery became popular in the 1970s by Tessier,⁴³ optic nerve damage was one of the most feared complications of craniosynostosis. Reports on the incidence of optic nerve involvement in craniosynostoses varied in the early studies, including as much as 100% for optic atrophy in oxycephaly in the 1900s,²⁹ and 54% for papilledema and optic atrophy in multiple craniosynostoses.⁷ In a study of 219 patients, Bertelsen⁴⁴ found a direct relationship between increased intracranial pressure with papilledema and optic atrophy, which suggested that the occurrence of optic atrophy is secondary to papilledema, and which was later confirmed in a twin study.⁴⁵ The onset of optic nerve involvement almost always occurred before the age of 7 years, and seldom progresses thereafter when cerebral growth is complete.⁴⁴ Further studies showed that although papilledema resolved with improvement of vision after craniofacial surgery, vision remained poor in patients with established optic atrophy.^{27,46} Decompressive craniofacial surgery to decrease intracranial pressure should be performed before the presence of optic atrophy, and preferably before there is evidence of papilledema or optic nerve dysfunction to ensure good visual outcome.

Based on our review, we found that corneal abnormalities occur in 24% of eyes, which led to visual impairment in 8% of patients with Apert's syndrome. Chronic corneal exposure secondary to proptosis compromises the ocular surface.^{18,20,21,28} Without adequate lubrication and lid protection, exposure keratopathy has been observed. Postoperative periorbital swelling and conjunctival chemosis and prolapse may worsen the corneal exposure due to altered tear film and inadequate lid closure over the compromised cornea.²⁸ Corneal ulcers and keratitis have been observed after fronto-orbital advancement and should be suspected early in patients who complain of red and painful eyes after craniofacial surgery. Generous amounts of ocular lubricants should also be routinely administered after orbital surgery. Taping of the eyelids at night and, if necessary, an intraoperative tarsorrhaphy may need to be done to temporarily protect the cornea.

The retrospective nature of our study design inevitably leads to shortcomings. Data obtained were incomplete, especially for refractions and visually evoked potentials, in which only 52% and 54% of the data were available. Seventy-four percent to 85% of data were present for all the other investigated parameters. As Apert's syndrome is a rare syndrome, the number of subjects in almost all parameters in this study may be large enough to produce representative findings. There is also probable ascertainment bias due to the referral of patients with more severe phenotypes to the unit.

The population of this study was characterized by the diversity of nationalities and an older age of presentation. Often the patients from Australia had annual ophthalmic follow-up as compared with patients from overseas, who

tended to have short-term ophthalmic reviews within the unit during the perioperative period. This disparity in the pattern of follow-up may affect the prevalence of ophthalmic findings.

In conclusion, visual impairment occurred commonly with Apert's syndrome, and amblyopia was the primary cause. Although optic atrophy was the major cause of visual impairment before the era of craniofacial surgery, the prevalence of optic atrophy is low under the current surgical protocols. This study showed that corneal damage also contributed toward visual impairment. Orbital surgery was complicated by corneal ulcer and corneal scarring, although intraoperative management has now been modified to prevent this from happening. Keratopathy and corneal scarring secondary to trichiasis were also observed. In order to achieve better visual outcome in patients with Apert's syndrome, early detection and adequate management of amblyopia is essential. Visual outcome could be further optimized by performing decompressive surgery before the presence of optic atrophy and by careful attention to protecting the cornea from exposure and trauma.

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Intellectual Outcomes Following Protocol Management in Crouzon, Pfeiffer, and Muenke Syndromes

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Abstract: Patients with craniosynostosis syndromes are traditionally managed by a dedicated craniofacial unit. Optimal long-term management of these anomalies is unclear, but in the Australian Craniofacial Unit, it involves ongoing care by an integrated multidisciplinary team, following a protocol that commences at birth and continues until the patient reaches skeletal maturity. The Australian Craniofacial Unit has, for the last 35 years, collected a significant series of patients with these conditions who have completed management from birth to maturity.

The aim of this study was to review this series of patients and assess the long-term outcomes of protocol management, focusing in particular on psychologic and social aspects.

This review demonstrates that these patients can do well in society, and many achieve higher education and find full-time employment. Regular follow-up and comprehensive multidisciplinary management allows for timely identification of any problems and appropriate intervention. This then helps to maximize the overall outcome for these patients.

Key Words: Craniosynostosis, crouzon, pfeiffer, muenke, syndrome, neuropsychology, outcome

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Management of patients with syndromic craniosynostosis traditionally takes place in a craniofacial unit. This entails an integrated multidisciplinary approach that is protocol-driven, begins at birth, and continues until the patient reaches skeletal maturity or beyond, if necessary. From its inception, the Australian Craniofacial Unit (ACFU) adopted a protocol-based approach to patient management. This has enabled us to examine the long-term outcomes of patients who have completed their treatment protocol and to assess the effectiveness of the protocol.

There have been few studies looking at the long-term outcomes in syndromic craniosynostoses, and most of these articles study morphologic outcomes.^{1–5} Those that include investigation

intellectual function are only in individuals or small numbers of patients with limited follow-up.^{6–8}

METHODS

A retrospective case note review of all patients listed in the ACFU database as having Crouzon, Pfeiffer, or Muenke syndrome managed through to maturity was undertaken. These patients were all managed within the framework of a protocol-driven multidisciplinary team approach. Not all patients were referred at birth, there being a significant number who were referred at a later age, mostly from interstate or as a part of the ACFU's overseas outreach clinical service. These patients were excluded from this study. Data were collected both from the notes and from the ACFU database. These data were entered into a spreadsheet program and analyzed.

MANAGEMENT PROTOCOL

The ACFU uses a protocol-driven approach to patient management from birth to maturity. For Crouzon, Pfeiffer, and Muenke syndromes, the ACFU protocol is outlined as shown in Table 1.

RESULTS

A total of 113 patients were identified on the ACFU database as having a diagnosis of Crouzon syndrome, 26 with Pfeiffer syndrome, and 5 patients with Muenke syndrome. Of these, 75 patients with Crouzon syndrome have reached skeletal maturity, 2 patients died before reaching maturity. Specifically, 14 patients with Crouzon syndrome have been treated from birth to maturity, and in addition, 14 patients with Pfeiffer syndrome have reached skeletal maturity, 7 of these have been managed from birth. There are only a small number of patients with Muenke syndrome identified, 4 have been managed from birth to maturity; 1 with a mild case of Muenke syndrome was referred at age 18 years but has had no surgical intervention. Two patients with Muenke syndrome in the series were twins.

Of the patients treated from birth to maturity, males outnumber females in all 3 groups, with an overall ratio of 1.8:1. However, when all of the patients with Crouzon, Pfeiffer, and Muenke syndromes who have been managed by the unit are considered, the numbers of male and female patients are similar.

SURGICAL PROCEDURES

The most common surgical procedure in all groups was fronto-orbital advancement, which was carried out in all but 3 of the 25 patients. Le Fort III osteotomies were carried out in 6 of 14 patients with Crouzon syndrome and in 3 of 7 patients with Pfeiffer syndrome.

There have been no major surgical complications in any of the patients with Crouzon or Pfeiffer syndrome. One patient

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TABLE 1. Management Protocol for Syndromic Craniosynostosis

Age	Protocol
Birth to 3 mo	Complete multidisciplinary assessment
3–6 mo	Planning meetings Surgery—priorities are airway, eye protection, and intracranial pressure Postoperative reviews
1 y	Total review Craniofacial clinic
1–10 y	Annual reviews (multidisciplinary) Dentistry (6 monthly checks) and orthodontic treatment Craniofacial clinics 3 yearly Surgery (if required)—priorities are airway, eye protection, and intracranial pressure
Teenage years	Annual reviews Dentistry (6 monthly checks) and orthodontic treatment Surgery—orthognathic, Le Fort III advancement Postoperative reviews
Late teenage years	Complete assessment Surgery—revision surgery Orthodontics (annual assessment, treatment, and prosthetic dentistry) Postoperative reviews Treatment complete

with Muenke syndrome lost sight in his left eye after frontofacial advancement.

MORTALITY

Of the patients in this series managed from birth, there have been 3 deaths before skeletal maturity. Two of the patients had Crouzon syndrome; the third was a patient with Pfeiffer syndrome. The cause of death of each patient was attributed to respiratory complications unrelated to surgery. All were females, and all were within the first 2 years of life.

One patient with Crouzon syndrome died after reaching skeletal maturity. This male patient had graduated from nursing college and was working as a nurse. Unfortunately, he had an unstable cervical spine and he died of a spontaneous subluxation of his atlantoaxial joint at the age of 19 years.

SOCIAL OUTCOMES

All patients seen at the ACFU are periodically reviewed by the social work team. Any potential social issues are identified and addressed. The patients in this series were all seen by a social worker; their final assessment was generally when they were in their midteens. Those patients who were from overseas were only seen by the social worker at their initial multidisciplinary assessment in Adelaide; the remainder of their follow-up being carried out in their home country.

The social work reports showed that all but 1 of the 25 patients in our series were from stable family backgrounds and

lived with their biological parents at the time of their final assessment. All of the patients had a supportive family structure, and most were noted to be coping well with their condition.

There was only 1 patient with Muenke syndrome who lived in foster care. He had a moderate intellectual disability and was also noted to have behavioral problems. Two patients, 1 with Crouzon syndrome and 1 with Pfeiffer syndrome, were noted to have signs of depression requiring further treatment at their final assessment.

None of the patients in this series are married or have any children yet. The mean age of the patients is, however, only 21 years; the oldest patient being 39 and the youngest patient is currently 16 years old. However, in our overall series of patients with Crouzon syndrome (113 patients), there are 7 who have married, 4 of who have children. A further 3 patients are not married but have had children of their own.

NEUROPSYCHOLOGY

As part of the management protocol, patients are seen by the neuropsychologists on a number of occasions to assess their development. The initial assessment occurs at 1 year of age. The child is then reviewed every 3 years until they are 10, followed by a final assessment at 15 years in most patients. Formal intelligence testing is done on at least 1 occasion in all patients. Patients who show signs of intellectual impairment (i.e., $IQ \leq 80$) are formally tested more frequently than those of average intelligence. These patients also receive extra assistance at school in the form of teacher aides and special classes. The nature of this extra input is not consistent between patients because the resources available vary considerably from state to state and between urban and rural communities. In all patients who had formal testing on more than 1 occasion, the results did not change significantly between tests, i.e., those that were shown to be borderline at 6 years remained borderline at 9, 10, 12, and 15 years.

The Wechsler Intelligence Scale for Children¹⁰ was used to assess children up until the age of 16, and the Wechsler Adult Intelligence Scale¹¹ was used if the patient was older than 16 years at the time of testing. Senior psychologists within our unit carried out the testing in all patients.

Of 14 patients with Crouzon syndrome, 11 have had formal neuropsychologic testing. Of the 3 patients not tested, 2 were from Oman and 1 was from Indonesia. These patients were not tested because they did not speak English and were only in Adelaide for a limited time before returning to their home country and also because of significant cultural differences that exist. The cognitive function was assessed in our patients using the age-appropriate Wechsler Intelligence scale.^{9–11} Of those that have been tested, 6 of 14 are of average intelligence, 2 are considered low average ($IQ = 80–90$), 2 are borderline for an intellectual disability ($IQ = 70–80$), and 1 patient has an IQ of 59, which is within the mild intellectual disability range.

Of the 14 patients, 10 are known to have completed secondary school. These patients all completed year 12 at 17 years. All these patients attended mainstream secondary schools. Those with average intelligence did not require any special education or teacher aides. Those patients who were identified as having learning difficulties all had access to a teacher aide and/or extra classes as appropriate. Of the patients who completed year 12, 3 patients have gone on to complete university degrees. Three patients are from overseas, and there was no information available about their educational level. One patient has started secondary school but has not yet completed year 12. When this group is excluded, 91% of patients with Crouzon syndrome managed from birth have completed year 12 at the same age as their peers. This compares very favorably with the general population (80% of students in Australia complete year 12).¹² Five

of the patients who have completed year 12 are too young to have completed tertiary education as yet. Of the remaining 2 patients, 1 did commence university but withdrew before completing his degree.

The patients with Pfeiffer syndrome seem to have a higher incidence of intellectual impairment. Of the 5 patients who have had formal psychologic testing, 2 are of average intelligence, 1 is described as being borderline for intellectual disability, 1 has a mild intellectual disability, and 1 is profoundly intellectually disabled. Two patients have completed year 12 within the normal time frame. One of these patients required a teacher aide and a significant amount of extra input to help her through. One patient is currently in year 11 at a mainstream school with no extra assistance. All but 1 of the patients has attended secondary school, 1 has obtained a bachelor of arts degree, and 1 has completed an apprenticeship as a motor mechanic.

All of the patients with Muenke syndrome in this series have had neuropsychologic assessment. The twins with Muenke syndrome have both completed year 12 on time, despite both being borderline for an intellectual disability (IQ = 70–80). They did require special education to help them through. The third patient is of average intelligence (IQ = 90–110). He did not require any special education and had no difficulties completing year 12. He is currently training to be a pilot. The final patient, who has a moderate intellectual disability (IQ < 70) has behavioral problems and has had several brushes with the law for assault and stealing. He did not complete year 12 at school despite having a significant amount of extra input.

Figure 1 summarizes the IQ of our patients from birth to maturity. The patients were tested at a mean age of 13 2/3 years. One patient was tested at 4 years and was found to be profoundly intellectually disabled.

EMPLOYMENT

Data on employment status of the patients in our series were incomplete. This reflects our finding that most patients tend to wait until their surgical treatment is complete before embarking on a career.

We do have some information on employment for 5 of the patients in our series. Of our patients with Crouzon syndrome, 1 is working as a chef. Another is a computer operator. One of our

patients with Crouzon syndrome works as a nurse, and another works part time in a supermarket. One patient with Pfeiffer syndrome is a pilot, and another is a motor mechanic.

DISCUSSION

To date, the ACFU has managed 14 patients with Crouzon syndrome, 7 patients with Pfeiffer syndrome, and 4 patients with Muenke syndrome from birth to skeletal maturity. A significant number of patients have been referred to the ACFU later in life. Of these patients, 60% were from within Australia and New Zealand. All of the patients seen in the ACFU were managed according to protocol.

Early intervention in fronto-orbital advancement is accepted as a key surgical component in the management of syndromic craniosynostoses^{1,3,4,13}; this procedure was carried out in all but 3 patients in our series. Le Fort III advancement to address the midface hypoplasia and faciotension is also well described in the literature^{1,2,4,6,14–16}; this was the second most common major procedure, being carried out in 6 patients.

In our series, all but 1 of our patients came from a stable and supportive family background and lived with their biological parents. Only 2 patients were found to show any signs of depression, which was managed appropriately.

For cognitive function, our results show that more than 50% of patients with Crouzon syndrome in this series have normal intelligence. Patients with Muenke and Pfeiffer syndromes were more likely to be intellectually impaired. A previous article from the ACFU⁶ showed that the IQ of patients with Crouzon syndrome was less than 70 in only 3 of 25 patients. However, these were not patients who had been observed from birth to maturity and had a median age of 9 years. Yacubain-Fernandes et al⁸ looked at the IQ of 11 patients with Crouzon syndrome between the ages of 16 and 156 months. They⁹ assessed the cognitive function in their patients using the Wechsler Intelligence Test Version 3. The IQ in their series ranged from 46 to 102 with a mean of 84.2. This is similar to our own findings.

The ACFU is a quaternary referral center, with patients being referred from both within the state as well as more complex cases from interstate and overseas. Consequently, it would be expected that the patient population that is seen is likely to be at the more severe end of the spectrum. This would be particularly true of the patients with Crouzon syndrome, which can have a very wide spectrum of severity. Most patients in this series have had transcranial surgery (29/32 patients). Whether this patient population has a higher incidence of cognitive impairment than more mildly affected patients requires further evaluation.

Three patients with Crouzon syndrome, 1 patient with Pfeiffer syndrome, and 1 patient with Muenke syndrome have successfully completed a university degree. All of the patients in our series have attended mainstream secondary schools, albeit with extra assistance in a number of patients; most have completed year 12. One patient with Pfeiffer syndrome did not make it further than grade 9, which he completed. One patient with Muenke syndrome did not complete year 12. This compares very favorably with the general school leaver population of Australia,¹² of which 80% have completed year 12.

CONCLUSIONS

Patients with syndromic craniosynostosis are complex patients who benefit significantly from integrated multidisciplinary care from birth to maturity. The surgery that is required varies and depends upon the severity of the condition. This generally involves cranial decompression early in life to prevent or alleviate raised intracranial pressure and/or fronto-orbital advancement to protect

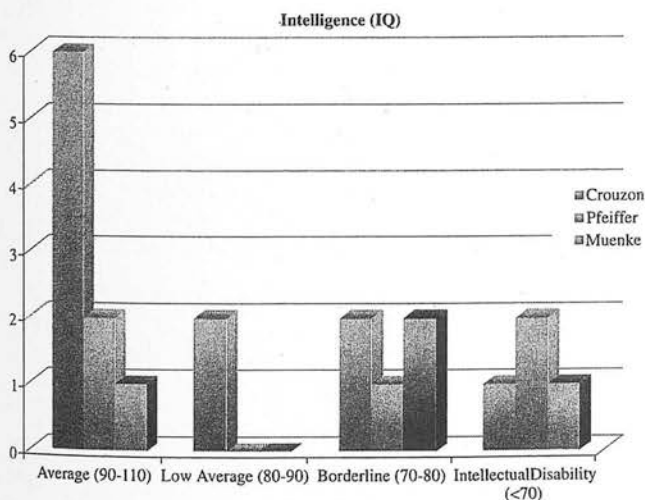


FIGURE 1. IQ in patients managed from birth to maturity.

the eyes. Once the patient is older, the midface may need to be advanced.

These patients can do well in society; many go on to achieve higher education and find full-time employment. There are a significant number of patients, however, who have specific neuropsychologic issues. Early identification of these as a part of a regular, protocol-driven, multidisciplinary management plan allows for timely identification of any problems and appropriate intervention. This then helps to maximize the overall outcome for these patients.

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Clinical Note

Late Results After Unicoronal Craniosynostosis Correction

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The authors report nine patients with unicoronal synostosis who were treated with the same surgical protocol and who now have reached skeletal maturity. These patients all underwent surgery at the Australian Craniofacial Unit during a 2-year period by one of two craniofacial surgeons and one of two neurosurgeons. The operative procedure in all of these cases was the same technique, in which there was unilateral advancement of the affected side. No cases required reoperation; however, one case subsequently required revision of the coronal scar, and two cases required strabismus correction. All cases were reviewed to evaluate patient results by the clinical staff; recent photographs at skeletal maturity also were reviewed. Two patients had adult computed tomography scans available for skeletal assessment, and one additional patient had undergone serial computed tomography scans during childhood. The patients also completed an anonymous questionnaire to ascertain their assessment of their appearance. The results of the clinical and radiologic assessments and questionnaire suggest that the operative procedure undertaken in these cases has produced satisfactory results in the long term, with few individuals requiring (or even considering) additional surgery, despite some persistent asymmetry.

Key Words: Coronal suture, craniosynostosis

Unicoronal synostosis is the early fusion of a coronal suture, which results in variable deformity in infancy. The deformity affects both the forehead and the face. This includes ipsilateral flattening of the frontal and pa-

rietal bones, temporal retrusion with elevation and recession of the supraorbital rim, and vertical orbital dystopia.^{1,2} The contralateral forehead may exhibit compensatory frontal bossing. The face may have deviation of the nasal root and even the chin.²

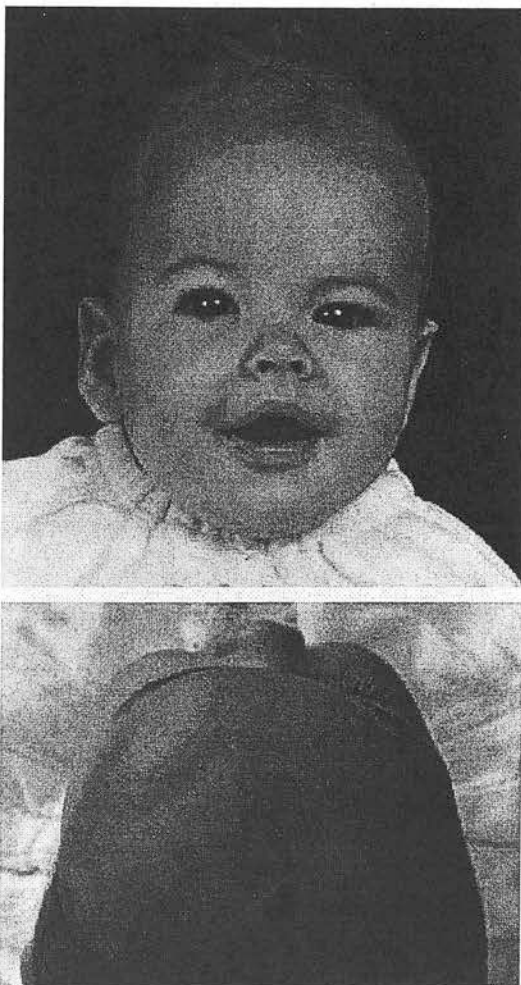


Fig 1 AP and vertical view of patient 1 before surgery, demonstrating the forehead and facial asymmetry.

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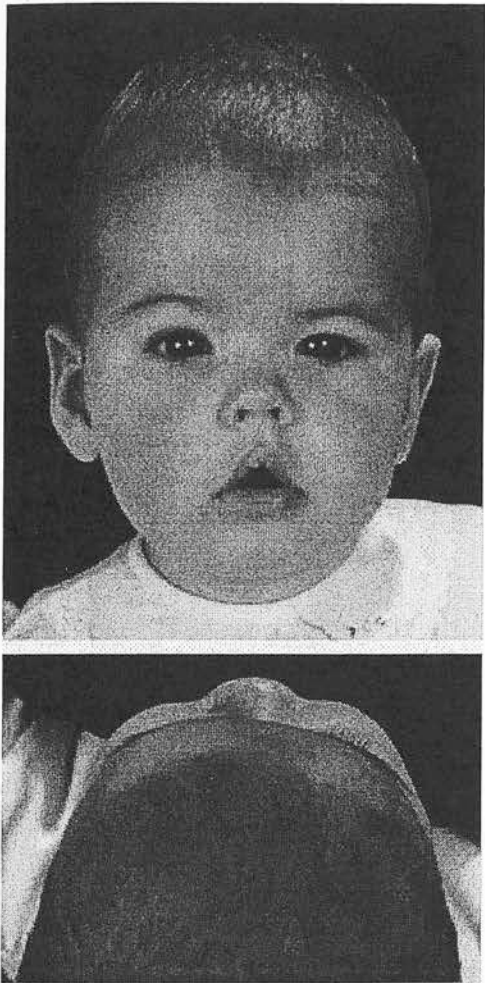


Fig 2 AP and vertical view of patient 1, 2 months after surgery, demonstrating the forehead symmetry.

Table 1. Summary of the Cases and Operative Procedures

Case No.	Affected Side	Sex	Age at Operation (months)	Additional Surgery	Syndrome
1	R	F	5	N	N
2	R	F	3	N	Y
3	L	F	3	N	Y
4	R	F	3	N	Y
5	R	F	11	Y	N
6	R	F	6	Y	N
7	L	F	3	N	N
8	R	F	2	N	N
9	L	M	3	N	N

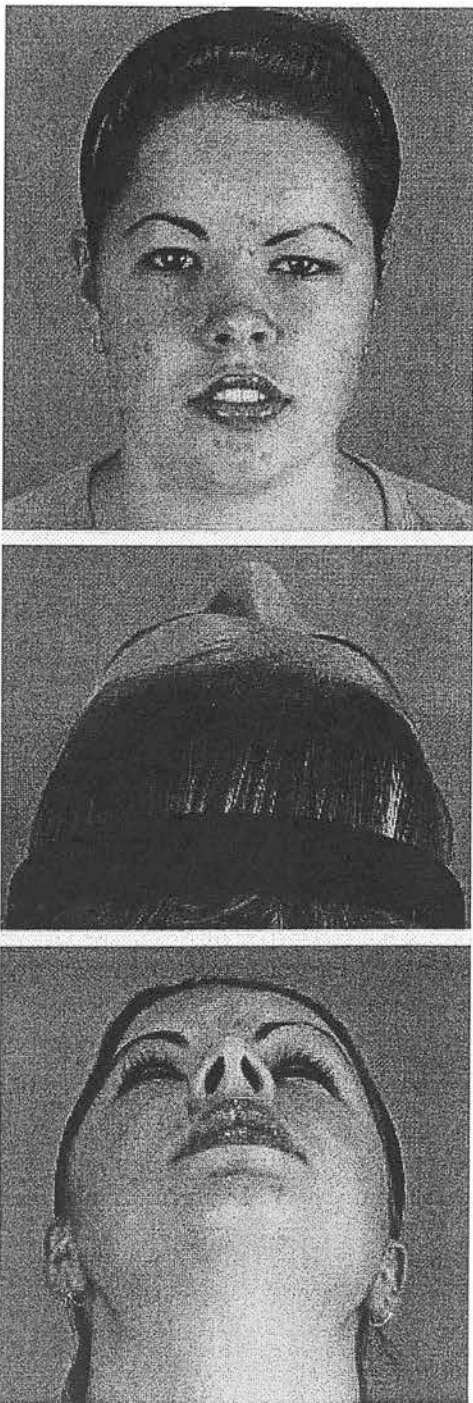


Fig 3 AP, Vertical view and worms eye view of patient 1 at age 19 years with no additional surgery.

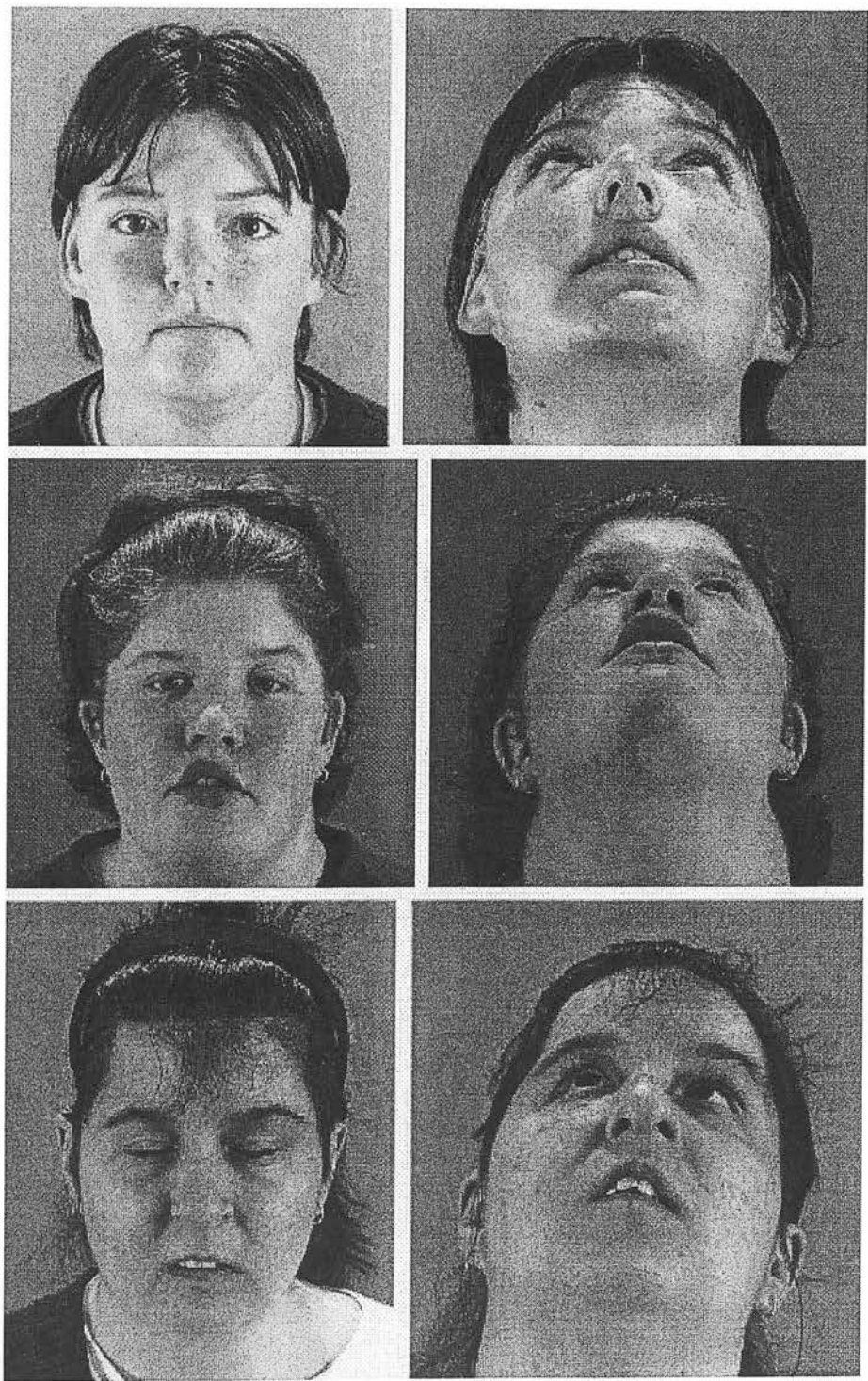


Fig 4 AP views of patients 2 to 9, supplemented with worms eye view.

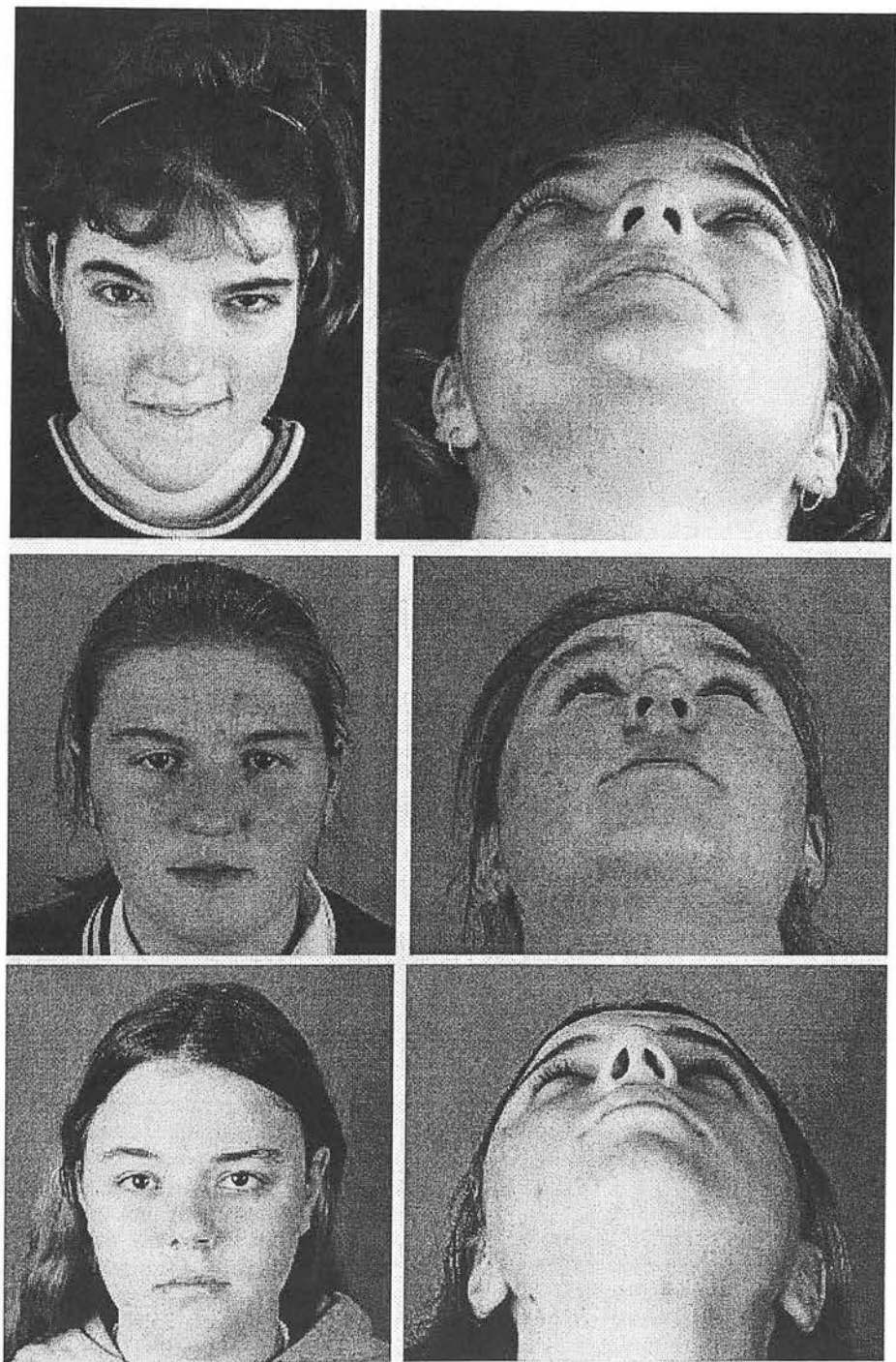


Fig 4 (continued)

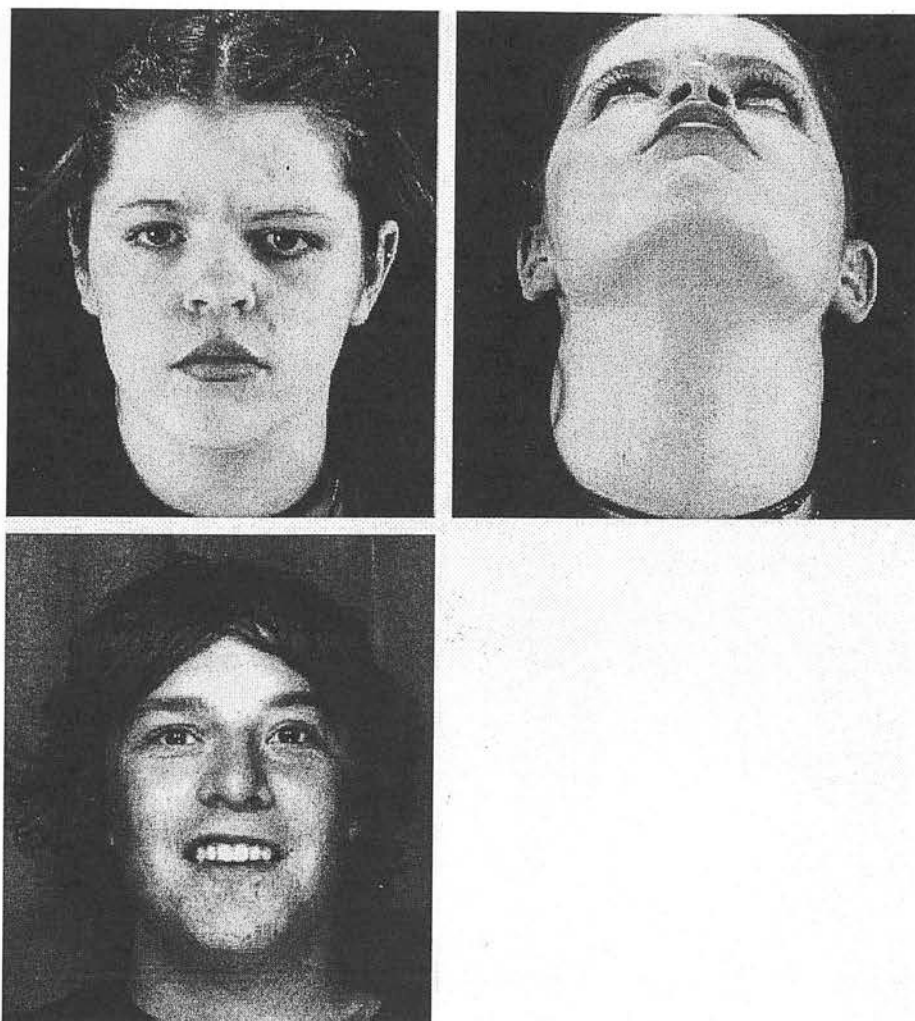


Fig 4 (continued)

The benefits of surgical correction have long been established,² and all operative corrections involve excision of the affected suture as the most important part of the procedure. However, the extent of additional osteotomies and reconstruction is a matter of debate—primarily as to whether only the affected side or both sides of the forehead require recontouring. There have been reports of results of both surgical approaches, but these have been limited by follow-up periods of a few years and well before growth is complete.²

We have undertaken a study of individuals who have been treated by the protocol established by the Australian Craniofacial Unit (ACFU), who have now

reached skeletal maturity, so we can assess the long-term outcome of our surgical intervention.

METHOD

All patients with unicoronal synostosis who have reached skeletal maturity were identified from the ACFU departmental database. They were invited to attend the department for review and clinical photographs or asked to supply a current photograph. All late postoperative computed tomography (CT) scans also were critically reviewed.

In addition, all of the patients were sent an anonymous questionnaire to ascertain their satisfac-

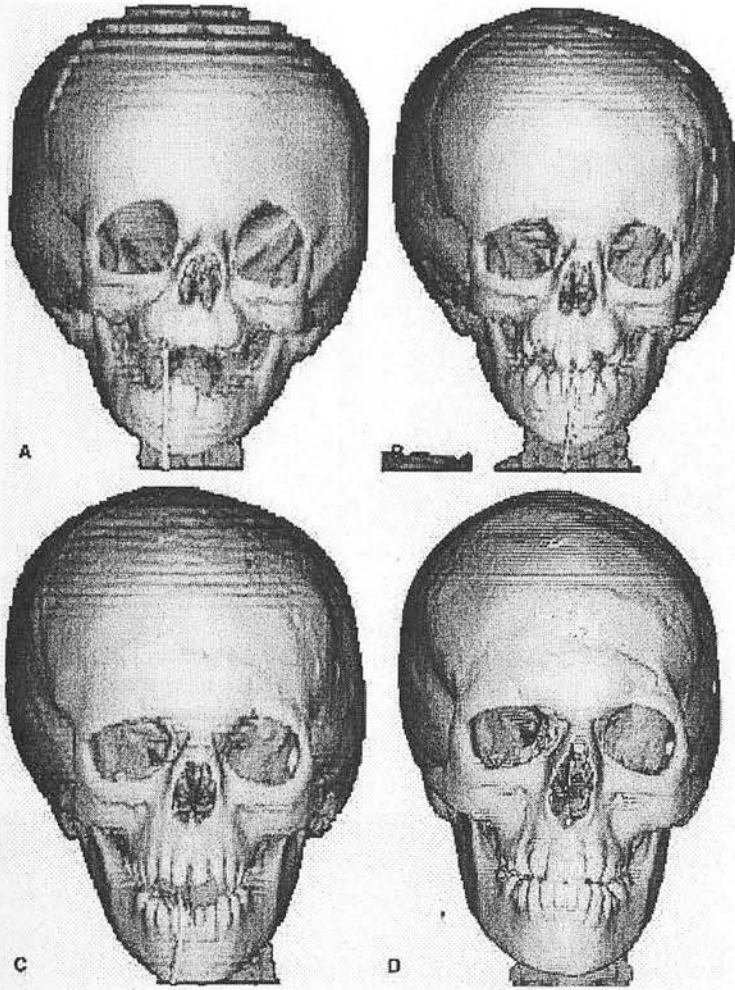


Fig 5 Serial CT scans of patient 6 (A) before surgery, (B) 2 weeks after surgery, (C) at age 4 years, and (D) at age 12 years.

tion with their final appearance, and whether any additional surgical procedures had been considered or undertaken to improve their appearance. The departmental case notes were reviewed to confirm the operative details and subsequent management in all cases, as were photographs and any later CT scans.

RESULTS

Nineteen cases of unicoronal synostosis were identified from the departmental case notes. It was confirmed that the operation was the same in all cases and consisted of removal of the affected coronal suture and unilateral advancement of the affected side. The follow-up to maturity at the unit was undertaken using the ACFU protocol, even when

early results were satisfactory (Figs 1, 2). The cases are summarized in Table 1. It is notable that of the patients available for study, only one was male, with the remaining eight being female. The age at operation ranged from 3 months to 1 year, although only two patients were older than 6 months.

The clinical assessment concluded that there was a range of cosmetic results (from very good to moderate), but all were deemed at least acceptable. The long-term photographic appearances are shown in Figures 3 and 4.

Two CT scans at skeletal maturity (patients 1 and 8) reveal that there was still some orbital asymmetry (Figs 5, 6), which was more evident than their respective clinical photographs suggested.

One additional patient (patient 6) had undergone three CT scans during childhood (Fig 7). These

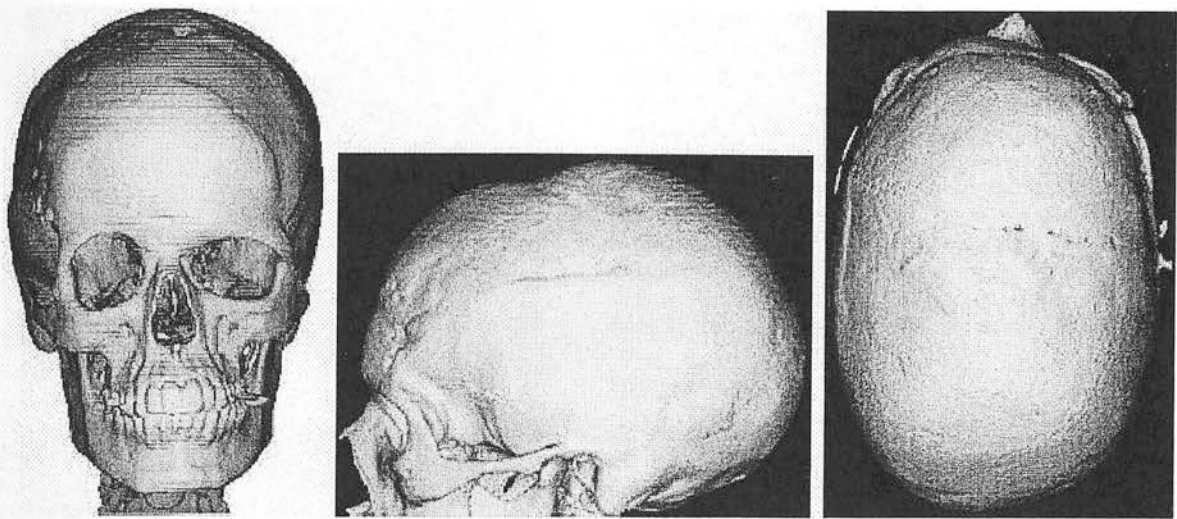


Fig 6 AP, left lateral, and vertex view of CT scan of patient 1 at age 18 years, with residual forehead and orbital asymmetry.

serial studies (along with the preoperative scan) underwent measurement of the intracranial volume using the Persona software package developed in our department and compared with normal values³ to

see if this changed with growth. The study of the intracranial volume of patient 6 showed that there were no significant changes with growth when compared with normal values (see Fig 8), which confirms that once the affected suture has been removed, the relative postoperative positions are maintained.

Replies to the anonymous questionnaire were received from all nine patients. These results revealed that they were all satisfied with their appear-

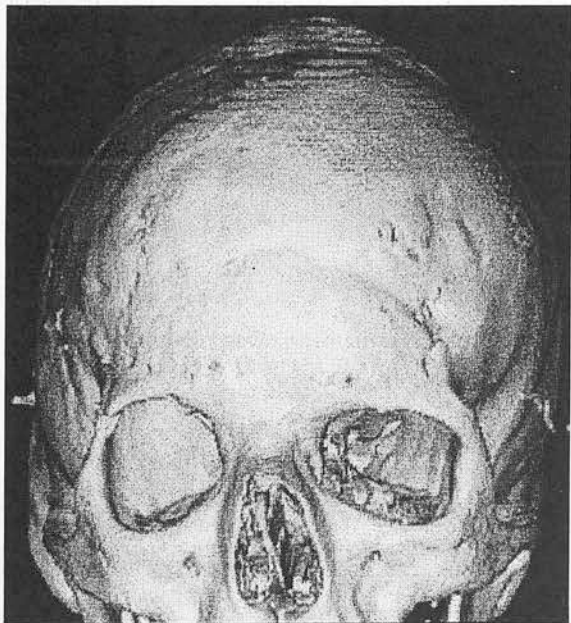


Fig 7 AP CT scan of patient 8 at age 17 years, with mild asymmetry.

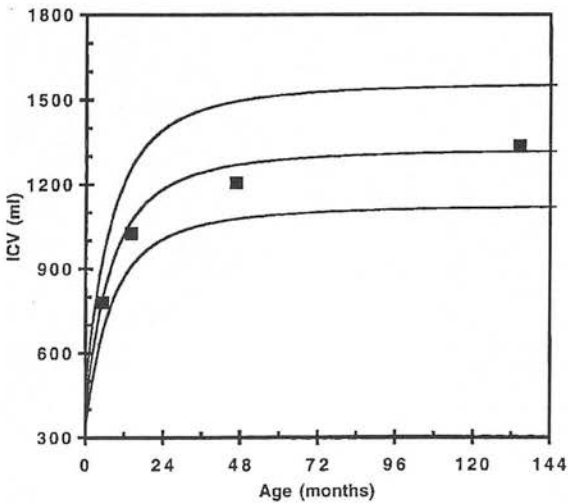


Fig 8 Case 6 showing the intracranial volume compared with normal values during growth.

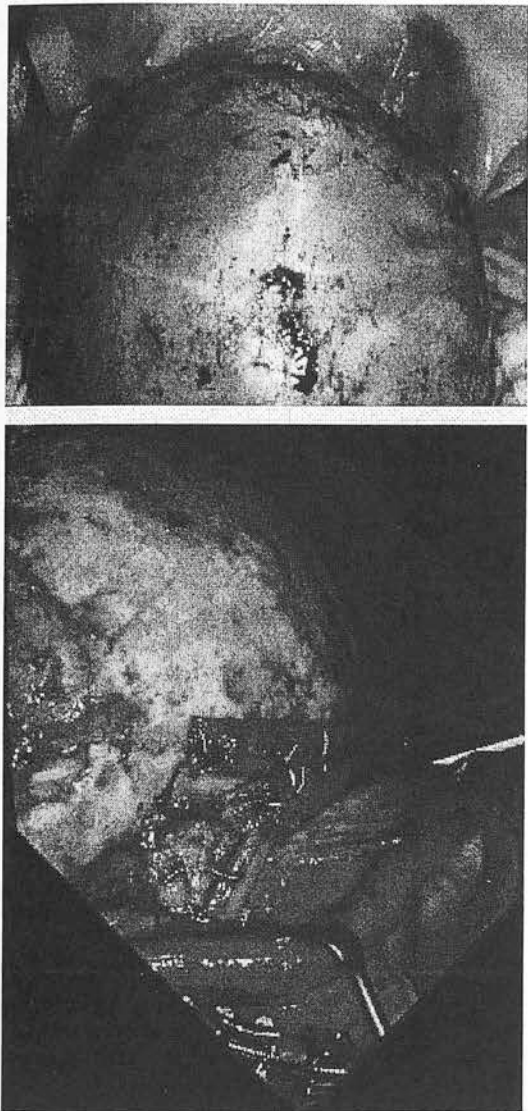


Fig 9 (a) Intraoperative view demonstrating the fused coronal suture affecting the right side. (b) Intraoperative view demonstrating the unilateral reconstruction.

ance, and no patient had undergone additional surgery elsewhere to improve his or her facial appearance. Interestingly, one patient (patient 3) was aware that her nose was asymmetrical but was not sufficiently concerned to consider additional surgical correction. This same patient also had been identified by the clinicians as having the least satisfactory result.

One patient (patient 8) had a stretched scar that was revised at the age of 14 years. Two patients had

consequences resulting from their abnormally shaped orbits on the affected side and experienced strabismus, which required corrective surgery (in one patient on two occasions). One other patient (patient 4) was offered ophthalmic surgery but declined.

All of these nine patients at the time of surgery were thought to have cases of nonsyndromic isolated unicoronal synostosis, but it has subsequently been found that three of these patients have the synostosis as part of a syndrome. These were Muenke syndrome (patient 2), Saethre-Chotzen syndrome (patient 3), and Hunter-McAlpine syndrome (patient 4).

DISCUSSION

The deformity produced by craniosynostosis of the unicoronal suture affects the forehead, orbit, and midface and occasionally the mandible. This produces a recessed brow and flattened forehead, along with a variable amount of orbital dystopia and contralateral frontal bossing.

Although the need for release of the affected suture is well established,⁴ the operation of choice remains uncertain. There are proponents of unilateral

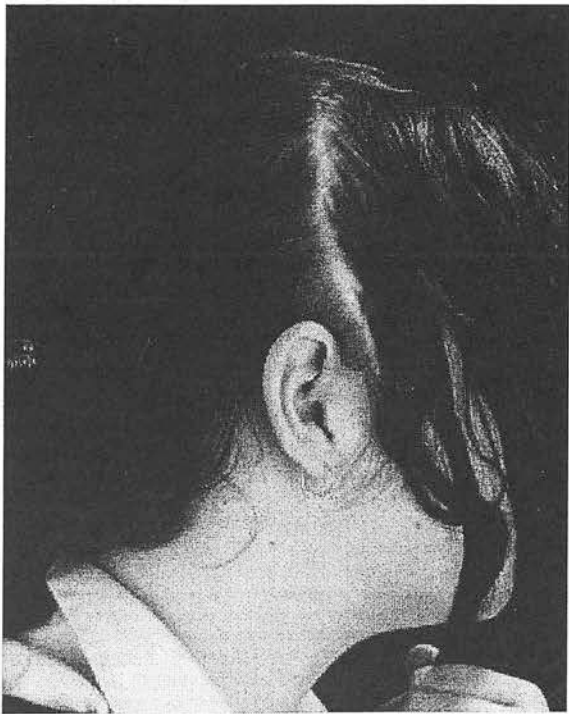


Fig 10 The widened scar on the affected side of the scalp of patient 8 at age 14 years.

fronto-orbital advancement,^{5,6} whereas others prefer a bilateral approach.^{7,8} Currently, direct comparison of postoperative outcome shows differences in outcomes, with both no discernible difference⁹ and better results with a bilateral approach being reported.² However, these postoperative results are at best only a few years after surgery, and long-term results of surgery for unilateral craniosynostosis are lacking. However, we believe that the outcome of any procedure cannot be fully assessed until skeletal maturity has been reached, when affected individuals may be more aware of their appearance and in particular of any remaining forehead, midface, or mandibular asymmetry.

The cases in this series have an unusual female-to-male ratio, when compared with a previous report of 2:1,² although most of the other 19 patients who could not be contacted were male.

All these patients underwent unilateral fronto-orbital advance in the manner described by the senior author¹⁰ (Fig 9). Our initial results from 11 cases were good with regard to the frontal recession and the orbital deformity, whereas serial radiographs also suggested improvement in basal deformity.¹⁰ Our initial results in 11 successive cases using this technique were reported at the RACS Scientific Conference in 1984 and were described as promising.¹¹

This is similar to the conclusion of a later report, which highlighted that asymmetrical synostoses treated before 1 year of age by unilateral orbital repositioning require no additional surgery in more than 90% of cases,⁵ although the mean period of follow-up in that series was less than 3 years.

We believe that critical examination identifies that there remains variable mild midface and mandibular asymmetry, particularly affecting the nose (which can best be seen on the worms eye view) and orbital dystopia. However, only one of our respondents (patient 3) mentioned this in the questionnaire or in response to direct questioning. This asymmetry is also highlighted in CT scans at skeletal maturity (Figs 5, 6).

There have been no patients who required reoperation and no examples of neurologic deficit or developmental delay, both of which have been reported.² No early complications, such as wound infection, cerebrospinal fluid leak, or even the later complication of prominent wires, are recorded. The late revision at age 14 years in patient 8 of the widened scar is the only notable complication (Fig. 10), and we already had modified our coronal scalp flap incision to reduce this risk.

The study of longitudinal changes in intracranial volume suggests that the relative postoperative proportions are maintained with growth.

The subsequent development of ocular problems (and in particular strabismus) in the reconstructed orbit in two of our cases (patients 7 and 8), are well recognized as late sequelae of unicoronal synostosis,¹² and this finding is similar to that of a previous report.² However, these are rarely reported in the craniofacial literature because they are managed by the ophthalmic team.

The subsequent identification in three of these cases that they had craniosynostosis as part of a syndrome reflects the improvements in diagnosis. It is significant that none of these patients had requested additional surgery because the outcome was still considered to be acceptable, although patient 3 had expressed some concerns regarding her nasal asymmetry earlier in childhood but currently is not wishing to pursue this further. It is particularly notable for patient 2, who was subsequently found to have an underlying FGFR3 mutation, because it has been suggested that this mutation is associated with a higher surgical revision rate.¹³

Finally, it is notable that two of the syndromic cases (patients 2 and 3) had some developmental delay, which was attributed to the underlying syndrome. None of the other patients experienced any speech or language delay that has been reported in unicoronal synostosis² and more recently on long-term follow-up of single-suture craniosynostosis.¹⁴

In conclusion, we have shown that the operation of unilateral frontal advancement for unicoronal synostosis can result in an adult who is unlikely to require additional revisional surgery, and in whom the result is acceptable to the individual and the surgeon. However, on the basis of the information that was currently available, this department changed its treatment protocol for unicoronal synostosis 12 years ago, and all subsequent patients now undergo a bilateral reconstruction, so there will be few additional cases available to add to this existing small study.

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RESEARCH ARTICLES

Breast Cancer Risk Is Not Increased in Individuals with *TWIST1* Mutation Confirmed Saethre-Chotzen Syndrome: An Australian Multicenter Study

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Saethre-Chotzen syndrome (SCS) is a rare autosomal dominant syndrome involving craniosynostosis, craniofacial abnormalities, and syndactyly. A recent Scandinavian study reported an increased risk of breast cancer in individuals with a clinical diagnosis of SCS. Because of the potential importance of this finding, we organized a multicenter study enrolling people with *TWIST1* mutation confirmed SCS to determine if an increased risk of cancer is present. This study did not identify any cases of breast or ovarian cancer in a cohort of equivalent power to that reported previously. These results provide clinical reassurance that at present there is no evidence for breast cancer screening above standard practice for individuals with SCS. © 2009 Wiley-Liss, Inc.

INTRODUCTION

Saethre-Chotzen syndrome (SCS) is a rare autosomal dominant craniosynostosis syndrome with variable expressivity of clinical manifestations including coronal craniosynostosis, ptosis, orbital dystopia, prominent crura, and minor syndactyly (Reardon and Winter, 1994). SCS is associated with heterozygous *TWIST1* germline mutations that can occur throughout the gene (el Ghouzzi et al., 1997; Johnson et al., 1998). The transcription factor activity of *TWIST1* is known to regulate the expression of members of the fibroblast growth factor receptor (*FGFR*) gene family (Rice et al., 2000) and mutations in the genes *FGFR1-3* have also been associated with a number of syndromic craniofacial syndromes including Muenke, Crouzon, Pfeiffer, Apert, and Jackson-Weiss (Jabs et al., 1994; Muenke et al., 1994; Reardon et al., 1994; Rutland, et al., 1995; Wilkie et al., 1995), which show some clinical overlap with the SCS phenotype.

TWIST1 expression has also been shown to regulate the activity of a number of genes with a central role in the development of cancer, includ-

ing *TP53* and E-cadherin (Stasinopoulos et al., 2005). Over-expression of *TWIST1* has been found to be a common finding associated with breast cancer progression and metastasis (Yang et al., 2004). A recent report appeared to link these two fields by reporting a markedly increased incidence of breast cancer in women with SCS (Sahlin et al., 2007). Fifteen women with breast cancer were identified in nine families among the 15 kindreds with a clinical diagnosis of SCS, an overall standardized incidence ratio (SIR) of 16.8. In the Scandinavian study, *TWIST1* mutation analysis was performed to confirm the diagnosis of SCS in only one of the 15 families and little information on the cancer histories in the wider families was available. Significantly four of the women with breast cancer and SCS

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were from a single family, but testing for other more common genetic forms of breast cancer predisposition were not reported. The reported increased risk of breast cancer reported by Sahin et al. would be highly significant if confirmed and would have major implications for the management of individuals with SCS.

To address this issue, we examined the risk for breast cancer and other malignancies in a cohort of *TWIST1*-mutation confirmed SCS families from Australia, ascertained through the craniofacial diagnostic testing program at the genetics laboratories of South Eastern Area Laboratory Services in Sydney. The study was designed to clarify the potential breast cancer risk by determining the frequency and distribution of cancer in the cohort of individuals with SCS and comparing this with age and birth-year matched population data.

METHODS

Ethics Approval and Patient Contact

Ethics approval for this study was obtained from South East Sydney and Illawarra Area Health Service, HREC Ref:POW 07/159. Families were ascertained on the basis of a diagnosis of SCS from the databases of the SEALS Molecular Genetics Laboratory, NSW, and Genetic Health Services Victoria. Referring geneticists and surgeons were contacted and asked to invite confirmed SCS patients to participate in this study. The initial patient contact was through the treating physician. Only the families with inherited mutations affecting adults were contacted: individuals with *de novo TWIST1* mutations in childhood without affected adults in a pedigree were excluded.

Study participants had provided consent for diagnostic molecular testing previously, and additional consent for the collection and use of clinical information including known *TWIST1* mutation status was sought from SCS individuals for the purpose of this study. Once consent was given, the proband for each family was contacted (or their parents where the proband was a minor) and family history of cancer was obtained using a standardized and previously validated questionnaire (www.kconfab.org.au). In addition, a number of clinical questions were used to investigate the possibility of SCS in other family members.

The data collected included demographic information (date of birth/death, sex), and cancer history (anatomical site and age at diagnosis for

each cancer) for the proband, as well as first-, second-, and third-degree genetic relatives of the proband. Additional questions related to the presence of features (craniofacial or cosmetic surgery, the presence of toe syndactyly, a history of deafness) that could suggest further, unrecognized cases, of SCS in the first-, second-, and third-degree genetic relatives of the proband.

Molecular Genetic Analysis

Mutation screening was performed either by denaturing HPLC analysis or DNA sequence analysis of the *TWIST1* coding exon in two fragments (*TWIST1* 359 bp and *TWIST1a* 461 bp). Patient DNA was prepared from peripheral blood collected in EDTA using a Qiagen DNA extraction kit, following the manufacturer's instructions and stored at 4°C at 100 ng/μL until used. Oligonucleotide primers (available on request) were designed for the *TWIST1* coding exon based on the *TWIST1* reference sequence (GenBank Accession number NM_000474). Patient DNA was amplified using PCR with the following constituents: 100 ng DNA, Platinum Taq 0.5U, 1.5 mM MgSO₄, 0.2 mM dNTP, 1x PCRx Amplification buffer, 5 pmol oligonucleotide primers. *TWIST1* is a GC-rich gene, therefore, the 3X PCRx Enhancer system (Invitrogen) was used routinely.

The samples were denatured and the Taq polymerase activated by heating to 95°C for 4 min, followed by 33 cycles of annealing at 60°C for 30 s, extension at 72°C for 1 min, and denaturation at 95°C for 30 s. The final cycle consisted of an extension at 72°C for 5 min. Denaturing HPLC was performed using a Varian Helix using the following denaturation temperatures listed in the table. DNA sequence analysis was performed using an ABI 3130xl Genetic Analyser with data analysis by Seqscape software. All analyses included amplicon-specific positive and negative controls. All mutations were characterized by bidirectional DNA sequencing.

Statistical Analysis

Individuals were censored at the age of death or their current age at the time of the questionnaire interview and carrier status was determined by molecular testing or clinical features consistent with a diagnosis of SCS in a relative of an individual with a known *TWIST1* mutation. An association between a *TWIST1* gene mutation and an increased risk of breast cancer was assessed by comparing, for mutation carriers, the observed

TABLE 1. Breast Cancer History and Family Mutation for 13 Families with Saethre-Chotzen Syndrome

Family	Affected		All		Mutation	
	No.	Br Ca	No.	Br Ca	cDNA	AA
1	28	0	93	0	c.230delA	K77X
2	5	0	18	0	c.421G>C	D141H
3	2	0	20	1	c.376G>T	E126X
4	2	0	21	0	c.190–200dup	S68T fs
5	4	0	59	0	c.395G>T	R132L
6	2	0	6	0	c.81–82delinsTT	Q28X
7	7	0	18	0	Chromosome 7 inversion ^a	
8	10	0	21	0	c.309C>G	Y103X
9	5	0	24	0	c.438–439insT	Q147X
10	3	0	12	0	c.779C>T	Q107X
11	3	0	14	0	Whole gene deletion	
12	4	0	8	0	c.92C>T	Q28X
13	14	0	31	0	c.376G>T	E126X
Total	89	0	351	1		

^aFamily 7 have an inversion of chromosome 7 with a breakpoint involving the *TWIST1* locus at 7p21. Typical SCS is the only phenotype observed in this family.

No., number of individuals; Br Ca, breast cancers; AA, amino acid; fs, frameshift.

number of females who had been diagnosed with breast cancer with that expected based on their age and year of birth. Year of birth, age, and history of breast cancer of relatives were ascertained from the proband and other family members. Where possible in the larger families more than one adult family member was interviewed (six individuals in family 1, two individuals in family 8); however, the data were not validated through population registries. The number of observed breast cancer cases and person-years at risk were calculated for calendar year. The expected number of cases of breast cancers in mutation carriers was estimated by multiplying the observed person-years by the age- and birth cohort-specific incidence rates of breast cancer from published national rates (Australian Institute of Health and Welfare, 2008). Breast cancer incidence rates prior to 1985 (when incidence data first became available for Australia) were estimated from US rates assuming the ratio of incidence rates in Australia to the United States before 1985 were the same as those after 1985. A SIR—the ratio of observed breast cancer to expected breast cancers—and its exact Poisson 95% confidence interval and corresponding *P* values were calculated using Stata (Stata 9, 2005).

RESULTS

Thirteen multiplex families with SCS were identified and all agreed to participate in the study. Mutations in the *TWIST1* gene were demonstrated in the proband in all families (Table 1).

These mutations included reported missense and truncating mutations, two of which were novel.

The cancer history of 351 individuals genetically related to the affected proband was obtained, which included 89 affected individuals with SCS (49 females). Thirty one of the affected women were more than 25 years old at the time of censoring. The median age was 34 years (range, 4–79 years) 43 years for the 31 adult females. None of these women were reported to have been diagnosed with breast cancer. One woman was reported to have been diagnosed with stomach cancer at age 78 years. There were no other reports of cancer. The expected number of breast cancers in these women given their age and birth year was 0.35 cases (95% CI 0–3.7 cases). The SIR was estimated to be 0 (95% CI 0–10.8).

Three cancers were reported in the 89 affected individuals, stomach cancer (diagnosed age 79 years), and two prostate cancers (both diagnosed at age 65 years). A further 8 cancers were reported in the 263 unaffected family members, including one breast cancer (diagnosed age 39), one colorectal carcinoma, three hematological malignancies, two stomach cancers, and a brain tumor.

Mutations in the *TWIST1* gene are described as having a high penetrance for at least some of the components of the SCS phenotype; however, we considered the possibility that a first-degree relative of an affected individual could harbor a *TWIST1* mutation in the absence of a recognized SCS phenotype. In the 13 families, there were 47 females untested for the family mutation, who were apparently unaffected first-degree relatives

of an affected individual, including 37 over age 25 years. In this apparently unaffected group of women, there was one diagnosis of breast cancer at age 39 reported in a deceased individual. This means that even if an undetected *TWIST1* mutation was present in any apparently unaffected first degree relative, it would not alter the finding of no evidence for an increase in breast cancer incidence.

DISCUSSION

We found no evidence for an increased risk of breast cancer in female carriers of mutations in the *TWIST1* gene in an Australian cohort of 13 families where the diagnosis of SCS had been confirmed by mutation testing. The *TWIST1* mutations demonstrated included missense, nonsense, insertion/deletions, and chromosomal disruptions to the gene, providing evidence that breast cancer is not associated with loss of function *TWIST1* mutations because of a number of different mechanisms. Given the size and the relatively young age of our sample of 49 SCS-affected women, we could confidently exclude a SIR higher than 10.8. The previous study of Sahlin et al. (2007) observed a SIR for breast cancer in women with SCS to be 16.8 (95% CI 1.5-32.1). Based on their reported 15 observed cases and 0.89 expected cases, the exact Poisson 95% confidence interval is 9.4-27.8. Therefore, the incidence of breast cancer in mutation carriers in our study was significantly lower than that observed by Sahlin et al. ($P = 0.014$). We believe that an increase in breast cancer risk of the of the magnitude reported by Sahlin et al. should have been apparent clinically given the number of families affected by SCS which have been seen through genetic and craniofacial clinics. The present study supports the clinical impression that breast cancer risk is not elevated in women with SCS secondary to *TWIST1* mutations.

In vitro studies also support opposing molecular roles for *TWIST1* in craniofacial development and breast cancer. In contradistinction to SCS, where *TWIST1* haploinsufficiency affects craniofacial development, *TWIST1* over-expression is associated with breast cancer (Firulli et al., 2005). Yang et al. (2004) found that *TWIST1* was strongly over-expressed in a mouse mammary tumor cells but not in normal mammary epithelium. Knocking-down the expression of *TWIST1* inhibited the proliferation and metastatic potential of the tumor cells. Subsequently, *TWIST1* over-

expression has been implicated in the progression and spread of breast cancer. *TWIST1* expression is also up-regulated in response to hypoxia-inducible factors (Gort et al., 2008) and promotes tumor angiogenesis (Mironchik et al., 2005). Subsequently, *TWIST1* over-expression has been associated with the potential for invasive and metastatic disease in a number of different cancers, including breast cancer. In addition, increased *TWIST1* expression has been associated with down-regulation of two well known tumor suppressor genes; *TP53*, which is mutated in the germline of a significant number of patients with Li Fraumeni syndrome (Stasinopoulos et al., 2005), and E-cadherin, germline mutations in which are associated with both diffuse gastric cancer and lobular breast cancer (Vesuna et al., 2008). *TWIST1* has also been shown recently to interact with the *TP53* DNA binding domain and to inhibit its function (Shiota et al., 2008).

Despite these in vitro findings, there are only two previous reports of malignancy associated with a *TWIST1* mutation in SCS. In the first case, a nasopharyngeal carcinoma was reported in a 32 years old with SCS (McKeen et al., 1984). However, in this case three siblings who were not affected by SCS were also diagnosed with cancer (hematological malignancies and testicular cancer) while a parent and 2 further siblings with SCS remained healthy. The second report involved a single individual with SCS with a diagnosis of renal cell carcinoma at age 5 years (Seifert et al., 2006). Interestingly, two germline *TWIST1* mutations were found on the same allele and the normal allele was apparently preserved in the tumor tissue. Finally, other components of the *TWIST1* effector pathway include the *FGFR2* and *FGFR3* genes, both of which have been implicated in cancer development: *FGFR3* as a bladder cancer suppressor gene and *FGFR2* in genome-wide association studies of breast cancer susceptibility (Easton et al., 2007; Lamy et al., 2006).

The number of multiplex SCS families with confirmed *TWIST1* mutations ascertained through the SEALS craniofacial diagnostic testing program provided an opportunity to address these conflicting strands of evidence on the role of *TWIST1* mutations in cancer by determining an accurate incidence ratio for breast cancer in this molecularly characterized Australian cohort.

It is not immediately clear why the Scandinavian and Australian cohorts of patients with SCS should display such markedly different breast cancer risks. It is possible that cases of breast

cancer in our families were unknown to the probands who provided the family history. However, previous studies have shown recalled breast cancer family history to be, on average, 90% accurate (Theis et al., 1994). In addition in the largest families in our cohort, the family history was ascertained independently through more than one individual (through three separate individuals for the largest family containing 28 affected individuals) with no inconsistencies reported. It is possible that some cases in the Scandinavian cohort could have an underlying mutation in a craniosynostosis gene other than *TWIST1* and it is this gene that is associated with an increased cancer risk. However, this explanation would be likely to account for only a small number of the excess cases and the one family in the report by Sahlin et al. that had a proven *TWIST1* mutation contributed four of the reported breast cancers. The authors did not report any investigations in their families for the known genetic causes of breast cancer, such as mutations in *BRCA1* and *BRCA2*, and it appears most likely that the difference in reported incidences is due to differences in the distribution of the heterogeneous causes of a genetic predisposition to breast cancer or sporadic cases confounding the analysis.

The principle objective of this study was to address the issue of whether women with *TWIST1* mutation confirmed SCS should receive specific advice on their breast cancer risk and be offered any additional form of screening. Some women involved in the study were already aware of the possibility of an increase risk. At least one Australian public health website dedicated to information about breast cancer and the OMIM entry 101400 have included information on an additional risk associated with SCS. Our study has not confirmed this additional risk and the information has been able to prevent unnecessary distress and as well as any additional investigations in the SCS families within our cohort. We, therefore, recommend that there is no indication to screen for breast or other cancers in people with SCS unless additional standard clinical indications are present, or other large cohorts with molecularly confirmed SCS with an elevated risk of breast cancer are reported.

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CHAPTER TWO

INTRODUCTION

Cleft lip and palate is the commonest of the craniofacial anomalies and is ubiquitous with an incidence of up to 1:600 live births reported¹. The more extensive facial clefts which affect the midface and the cranial vault as originally described by Tessier are much rarer. Curiously, unlike other congenital craniofacial anomalies there is an uneven geographical distribution of those with extensive midline facial clefts with these occurring more commonly in SE Asia and the Pacific basin than in European and American populations. The reason for this remains uncertain with genetic and nutritional factors cited as possible causative factors². However, some Tessier clefts are part of syndromes of which Treacher-Collins syndrome is best known, where the anomaly is known to result from an underlying genetic mutation of the "treacle gene" on chromosome 5³.

So this group of clefting conditions the factors controlling the underlying disease processes and the variable expression requires elucidation. The management of non-syndromic cleft lip and palate is now by protocol management in major centres but for the rarer facial clefts it is often determined on the basis of a few cases and small series, with outcomes at skeletal maturity rarely considered.

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CHAPTER TWO - PAPERS

The papers in this chapter review investigations of both cleft lip and palate and Tessier facial clefts. The non syndromic cleft lip and palate papers are presented first.

The first paper investigates the multiple underlying genetic factors which are thought to be involved in cleft lip and palate. Multiple genes and epigenetic factors are thought to be involved in the production of non-syndromic cleft lip and palate. It has also been established that dental anomalies are common in cleft lip and palate patients. This study investigates the dentition of phenotypically normal parents (ie no clefting present), of children with clefts of the primary palate to identify if there are dental anomalies. The study investigated whether these could act as a marker for risk in producing an offspring with a cleft. The study failed to identify any association but it suffered from the weakness in that it was a small study, which with retrospective review may not have been powerful enough to answer the study question adequately.

The second paper was a study to investigate the incidence of middle ear disease in children with a submucous cleft palate. Although the palatal mucosa is intact, the levator and tensor muscles are incorrectly orientated, which may impact on eustachian tube function and predispose to the development of middle ear disease. This is a group of

children often experience difficulty in their diagnosis being made by clinicians at an early age allowing appropriate management and timely interventions. Those presenting late may have irreversible deafness, even among those living in western metropolitan cities. The outcome of this study found that the incidence of middle ear disease was similar to children with unilateral cleft lip and palate, with over 90% requiring ventilation tubes as part of management. This finding has important implications for management of children with submucous cleft palate.

The third paper is a case report which describes a late complication of arterio-venous shunting following Orthognathic surgery in a patient with midface hypoplasia associated with cleft palate. The incidence of such complications in the cleft population was reviewed and the subsequent management with the use of interventional radiology is described.

The fourth paper is a radiological study describing the spinal anomalies in Goldenhar syndrome. Although previously spinal anomalies of the cervical spine had been well described – they can be seen on cephalograms which will be regularly ordered by treating Orthodontists, this study reviewed the whole length of the spinal column and found there were a significant incidence of anomalies in the thoracic and lumbar spine as well as in the cervical region. The implications of these anomalies for clinical management are described.

The fifth paper is a case report in which a description of changes in the operative surgical technique which result due to the different

anatomy which occurs in the mandible in those who have undergone costochondral graft reconstruction of the mandibular condyle and ramus for hemifacial microsomia.

The sixth paper is a detailed study investigating the clinical features which can occur in one of the commonest syndromic forms of cleft lip and palate, Van der Woude syndrome. Unlike most forms of cleft lip and palate this is known to result from the mutation of a single gene. Surprisingly, in studying affected families a full range of cleft conditions were found from bilateral cleft lip and palate to submucous cleft palate. This highlights the critical role that other genes and epigenetic factors may have as a full spectrum of cleft lip and palate phenotypes can arise from a common single genotype.

The seventh paper is a review of a complex syndrome (Opitz G BBB syndrome) with both cleft lip and palate and craniosynostosis. This is a relatively rare condition due to anomalies in the Hox genes controlling midline development of the body. Previously there have been mainly case reports, whereas this study reviewed the phenotypes (along with management) of a group of patients. The paper highlighted some of the anatomical variants which impact on surgical technique. Some cases in the series had reached skeletal maturity allowing a limited long term review of outcomes.

The eighth and ninth papers are the first published multidisciplinary review of outcomes at skeletal maturity in children with isolated cleft

palate and bilateral cleft lip and palate respectively, who had been managed by protocol and operated by a single surgeon. These not only considered the aesthetic outcomes, but considered orthodontic (growth), otolaryngological (hearing) and speech. These papers have encouraged other cleft surgeons to measure and report their own results in a similar manner. They have raised awareness among surgeons and promoted a cultural change and attitude in that outcomes should be assessed at skeletal maturity rather than a few years after a surgical intervention.

The tenth paper is a longterm outcome study of the first reported case of Treacher Collins syndrome who underwent mandibular distraction to allow removal of his tracheostomy at five years of age. This paper highlighted that the benefit in airway improvement was only temporary and although the tracheostomy wasn't re-opened, the dysmorphic facial growth pattern supervened requiring the respiratory physicians to prescribe CPAP at night to maintain adequate oxygenation. The other important finding was that mandibular distraction in childhood didn't avoid the need for bimaxillary orthognathic surgery at skeletal maturity. This study helps define the role for distraction osteogenesis as part of protocol management of Treacher-Collins syndrome.

The eleventh paper is the largest published long term outcome study of Treacher Collins syndrome patients who have been managed by a treatment protocol. This review has been used by the International

Society of Craniofacial Surgeons as the basis of its “ benchmarking” of International standards of care of this condition.

The twelfth and final paper in this chapter is a longterm review, at skeletal maturity of cleft patients who required revisional surgery after undergoing pharyngoplasty during childhood. It demonstrated that in some children it may become redundant and require taking down, while in others it may require repositioning for optimal functioning as a result of differential growth of the pharynx. It highlights the need for long term follow up in all children who undergo a pharyngoplasty procedure.

In summary these papers extend the knowledge of the clinical features of children with clefts of the facial skeleton, promotes protocol management and reviews long term outcomes.

Dental Findings in Parents of Children with Cleft Lip and Palate

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The incidence of dental abnormalities in the cleft lip and palate population has been reported to be much higher than in the normal population. The role of genes in the production of a cleft lip and palate, and dental anomalies is thought to be complex, with autosomal dominant, recessive, and x-linked genes all playing a role. Noncleft parents can carry some of the cleft lip and palate genes, which produce clinically subtle manifestations in their facial skeleton. The purpose of this study was to look for evidence of increased dental anomalies in the noncleft parents of cleft lip and palate children. The dentitions of the parents of 60 children with different types of cleft lip and palate were examined prospectively to see whether or not they exhibited features found more readily in the cleft lip and palate rather than did the normal population. Their dentitions were studied to record the following dental features: congenitally missing teeth, supernumerary teeth, or morphologic changes of the crowns of the permanent teeth. The number and position of any frenal attachments were also recorded. The results of this study did not show any differences in incidence of dental anomalies from the noncleft population. There was no evidence to support the hypothesis that congenital absence of lateral incisors is a microform of cleft lip and palate. Further, these results also failed to reveal any consistent pattern in the number and position of frenal attachments.

KEY WORDS: *dental anomalies, dental morphology, microform cleft*

There are many different mechanisms that result in the clinical manifestation of cleft lip and palate: some are caused by single mutant genes, some are due to chromosomal aberrations, and some are caused by specific environmental agents. It is probable that the great majority are caused by the interaction of genetic and environmental factors, each with a relatively small effect. A wide range of visceral and skeletal anomalies are found in association with cleft lip and palate embryos, and the incidence of these is higher than in noncleft embryos. The action of the clefting genes produces an underlying disturbance in a number of body tissues including the dental lamina (Johnson, 1967). This is thought to be the result of malfunction of the neural crest cells (Sharma and Kharbanda, 1991). Some of the genes involved in the production of a cleft lip and palate are carried by asymptomatic parents. Indeed, it has been suggested that a substantial genetic component may be present in at least one parent (Ward et al., 1989).

There is mounting evidence to suggest that there are differences in the facial skeleton of the parents of all types of cleft lip and palate patients compared with the rest of the noncleft

population. These differences have been explained as the result of action of some of the genes that are important in producing clefting in their offspring (Coccaro et al., 1972; Kirusu et al., 1974; Nakasima and Ichenose, 1983; Raghaven et al., 1994).

It is well established that the dentitions of those with cleft lip and palate differs from that found in the noncleft population (Bohn, 1963; Delude and Payette, 1991a). These differences include a higher incidence of congenitally missing teeth (Olin, 1964; Ranta, 1983). These have been reported as occurring in 24% of cleft lip and palate cases (Olin, 1964). The tooth most commonly missing in the cleft population is the second maxillary premolar, but all of the other premolars and the upper lateral incisors are affected in the permanent dentition. A higher incidence of supernumerary teeth (Millhon and Stafne, 1941) and alterations in the dental morphology have also been reported (Bohn, 1963). At least 15 dental morphologic abnormalities have been reported as occurring at a higher incidence than in the normal population (Kraus et al., 1966; Jordan et al., 1966). These include differences in both the primary and secondary dentitions, but only those affecting the permanent dentition in adulthood will be considered in this paper. These morphologic variations include further variations of the permanent lateral incisor, which may be T shaped or have a palatal cusp. Other anomalies include an absent cusp or altered cusp patterns on the maxillary first permanent molar, the mandibular first premolar, and mandibular second premolar, and the addition of parabolabial tubercles on the central incisor and the canine.

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The hypothesis that the possession of some of the cleft genes by a noncleft parent may result in clinical manifestation within the dentition is supported by the observation that parents of several cleft lip children exhibit congenital absence of the permanent upper lateral incisors (Lucas, 1882). This led to the suggestion that the absence of an upper lateral incisor may represent a microform of cleft lip and palate. A previous study into the dentition of the parents of those with cleft lip and palate limited the investigation to whether or not a single tooth, the upper lateral incisor, was present, absent, or exhibited only one morphologic abnormality, namely "peg" shape (Woolf et al., 1965).

To establish the incidence of dental anomalies in the asymptomatic parents of those with cleft lip and palate, a prospective study was undertaken. Both parents completed a questionnaire to elucidate a detailed dental history, after which a mirror-and-probe dental examination was performed.

METHOD

Asymptomatic parents of successive cleft lip and palate patients who were admitted to the plastic surgery wards over an 8-month period were asked to participate in the study. Although none refused, 10 patients had to be excluded from the study because examination of both natural parents proved to be impossible. Another case was excluded because the father possessed a cleft lip. Assessment of the natural dental morphology of all parents was possible.

Parents completed a questionnaire to ascertain both the family history of cleft lip and palate and their own dental history, particularly regarding any additional, missing, or extracted teeth. We anticipated that this would reveal whether or not any supernumerary teeth (including mesiodens) had been extracted. The questionnaire was also used to establish the original morphology of the permanent teeth prior to their undergoing any restorative procedures and extractions. Patient questionnaires were used to elucidate dental histories, because many parents had attended for dental care infrequently in the past and had often presented to several practitioners. Successful retrieval of meaningful records from a large number of dentists was not possible. Dental examination using a mirror and probe was performed by a single observer (P.A.). The number, position, and morphology of all teeth, and also the position and number of frenal attachments were recorded.

The parent population was divided into three groups depending on whether their child had a cleft lip only, a cleft palate only, or both cleft lip and palate.

RESULTS

The parents of 60 patients were included in the study. These were divided into the following groups according to their child's condition: cleft lip only (19), cleft palate only (14), and cleft lip and palate (27). The age of the patients ranged from 2 days to 15 years, with a median age of 7 months. The sex ratio of the children was 41:19 (m:f). The age of the parents

ranged from 17 years to 48 years, with a median of 23 years. Four of the parents reported having other family members affected with cleft lip and palate. The dental histories were mostly unremarkable, with only one parent who claimed to have had two supernumerary incisors removed as a child. (Whether these represented supplemental lateral incisors or mesiodens was impossible to ascertain as the patient had changed dental practitioner and previous records had been lost.)

Dental examination of the parents revealed that (apart from wisdom teeth) only 36 teeth were missing or extensively restored, so it was impossible to determine the natural dental morphology. These missing teeth were nearly all first molars or first premolars; a history of extraction as part of orthodontic treatment or for caries was obtained for 31 extracted teeth. Four teeth had crowns (three upper central incisors and one lower second premolar). One upper second premolar was absent in one parent [1/120 (0.9%)]. This occurred in a mother of a unilateral cleft lip child, and as there was no history of extraction and, as it was also absent on a dental radiograph, it was presumed to be missing congenitally.

Careful examination of the dental morphology of the upper lateral incisors revealed one parent to have bilateral "peg" teeth. All other upper lateral incisors were judged to be within the normal range, and specifically, no cases of a palatal cusp or a "T" palatal ridge could be found despite careful search. There was no case of the reported cusp abnormalities affecting the maxillary molars, or mandibular first or second premolars. No paralabial tubercle was identified on any tooth in this population.

Examination of the position and number of the frenal attachments revealed that all parents had at least one frenulum and that this was universally present between the upper central incisors. However, seven parents had two frenula and one had three. The positions of these additional frenal attachments was inconsistent, with mothers and fathers of all groups affected.

DISCUSSION

This population studied is thought to be representative of different types of cleft lip and palate in the U.K. population. All cases where the affected child had a cleft lip and palate as part of a syndrome associated with other anomalies were excluded, as it has been shown that those with syndromes can have a higher incidence of dental anomalies (Delude and Payette, 1991b; Baccetti, 1993). The parents used in this study had their natural permanent dentition intact or at a level of restoration that did not obliterate the natural morphology. However, the young age of the cleft offspring precluded an examination of their permanent dentitions to compare with their parents.

The results of this study were that only a single tooth was found to be congenitally missing, and no supernumerary teeth were present. This supports the view that the number of teeth in the parents of all types of cleft lip and palate are the same as the noncleft population. However, differences in the incidence of dental anomalies has been reported between those with cleft palate and those with both unilateral and bilateral

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MIDDLE EAR DISEASE IN CHILDREN WITH SUBMUCOUS CLEFT PALATE

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Objectives: The incidence of otitis media with effusion (OME) in those with submucous cleft palate (SMCP) is currently unclear with few published studies worldwide. We wished to establish the incidence in an Australian population.

Method: Twenty cases of SMCP who had reached the age of twelve years underwent case note review to establish the incidence of middle ear disease of sufficient severity to warrant surgical intervention.

Results: 12/15 cases who had their cleft diagnosed by five years of age required at least one surgical intervention, while none of the five cases presenting after twelve years of age required intervention.

Conclusion: OME would appear to be very common in young children with SMCP and we recommend that all children with this diagnosis undergo regular specialist Otolaryngological assessment.

Key words: Submucous cleft palate, Otitis Media

Introduction

Submucous cleft palate is a cleft of the palate where the palate appears at first glance to be intact, but the underlying velar muscles are abnormally orientated (Figure 1). Specifically, the levator muscles are inserted into the back of the hard palate rather than meeting each other in the middle to form a sling. The term smcp was coined in 1910¹ and the condition is characterised by bifid uvula, notching of the hard palate and with lucency in the midline of the soft palate, also known as Calnan's triad². Because it may appear that the soft palate is intact, the diagnosis may be delayed, and occasionally, may not be established until adulthood.

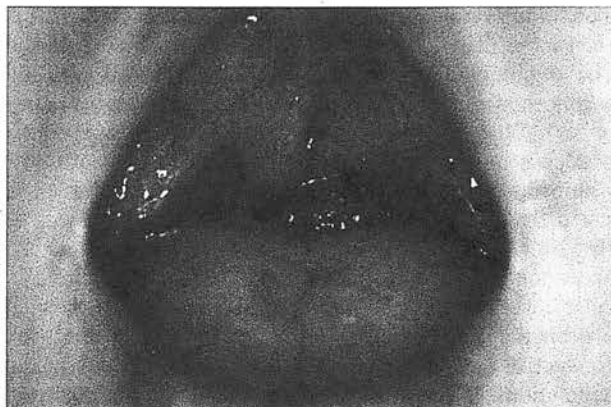


FIG. 1. The appearance of a submucous cleft palate demonstrating the obvious bifid uvula in this case.

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In a complete isolated cleft palate the risk of OME is now well established since the initial reports³, as are the benefits of management with ventilation tubes⁴. However, there are currently few published studies relating OME in those with SMCP. This retrospective study attempts to clarify the relationship between OME and SMCP, although this is limited by the small number of cases.

Method

The departmental database was used to identify cases where all the information was available for study. The case notes were reviewed and the patient details and any surgical interventions recorded.

Results

Twenty cases were identified from the departmental database. Fifteen cases were identified who presented for

management of their cleft before ten years of age and five cases were older than this at the time of presentation. Eleven were males and nine were females.

In the younger group 12/15 had recurrent middle ear disease requiring the insertion of ventilating tubes on at least one occasion (see Table 1). Of these, four cases eventually required long-term ventilation with T-tubes. Two cases required tympanoplasty.

Table 1.

Number of Operations for Grommet Insertion in SMCP Patients

No of Operations (Range 0 - 4)	0	1	2	3	4
No of Cases (Total = 15)	3	5	3	3	1

However, of those five cases presenting at an older age i.e. over ten years of age, none required ventilation tubes.

Discussion

SMCP is a thought to be rare, but because of the difficulty in making a diagnosis, it is certainly under reported. This is even more difficult in the occult form where the classical triad of clinical features are absent but the muscle insertion is abnormal⁵. In one screen of 10 000 children an incidence of 1:1,200 of SMCP has been reported⁶, while the incidence of occult form is unknown. The extent of OME within the SMCP group remains unclear and it has been stated that it awaits evaluation with a prospective study⁷. However, the recognition that OME can occur is well understood with palatal muscle malformation resulting in velar insufficiency and the resulting in Eustachian tube malfunction combining to produce otitis media and conductive hearing loss in the normal and cleft palate populations^{8,9}. The significance of OME is that recurrent infection has been used as one indication for repair of the submucous cleft¹⁰.

The findings of this study with its younger presenting age group of 12/15 cases (87%) requiring surgery on at least one occasion, suggests that OME is in fact common among SMCP cases. The absence in the older presenting cases could be due either to them having a milder cleft (occult form) or OME at a younger age. However, the small numbers in this study only allow speculation on this point.

These results when compared with previous studies of SMCP which have found ear problems (not specifically defined) in 55%⁶, and middle ear disease in 32% (16/47 cases)¹¹, suggest that the incidence in Australia is higher than the current literature suggests. What is particularly notable from our results is that almost half the children (7/15 cases) in the younger group required surgery on two or more occasions and in addition four cases required T-tube insertion for chronic difficulties, suggesting that the middle ear disease can be persistent.

In conclusion we have shown that the incidence of OME in SMCP in an Australian population is higher than previous studies and that when it occurs, the problem can be significant for many cases. These results highlight the need for regular Otolaryngological assessment of children with SMCP.

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Traumatic Arteriovenous Malformation Following Maxillary Le Fort I Osteotomy

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Objective: Complications following maxillary Le Fort I osteotomy are rare. The authors present the rare complication of an arteriovenous malformation following such a procedure in a 25-year-old woman with a cleft lip and palate that was treated successfully with radiologically guided embolization.

KEY WORDS: arteriovenous malformation, embolization, maxillary osteotomy

The Le Fort I maxillary osteotomy is a commonly undertaken procedure for the correction of dentofacial discrepancies. This is particularly so in those patients with a cleft lip and palate in whom there may be an associated retrusive or hypoplastic maxilla. Orthognathic surgery for patients with cleft lip and palate varies in different centers, but it has previously been reported that up to 32% of patients with unilateral cleft lip and palate will require either a maxillary osteotomy or bimaxillary surgery (Schnitt et al., 2004). Complications following Le Fort I maxillary osteotomy are rare. The most common complications are hemorrhage, infection, ischemia, temporomandibular joint problems, and relapse. We present an exceptionally rare complication of traumatic arteriovenous malformation following Le Fort I osteotomy and its subsequent management.

CASE REPORT

A 25-year-old woman with cleft lip and palate underwent an advancement Le Fort I maxillary osteotomy and sagittal split mandibular set back osteotomy. Figure 1 shows the preoperative lateral cephalogram. The maxillary osteotomy was carried out uneventfully. There was no unusual hemorrhage at the time of pterygomaxillary dysjunction, and the maxilla was advanced 8 mm without difficulty. At the completion of surgery, the patient was transferred to the ward, where she made a good recovery prior to discharge from the hospital 2 days later.

The patient was followed up regularly in the outpatient department. She was asymptomatic until 3 months after her sur-

gery, when she complained of a left-sided pulsatile tinnitus. She was therefore referred to an otolaryngologist, who scheduled a computed tomography scan. This demonstrated a large, convoluted niche of abnormal vessels within the left infratemporal fossa involving the left pterygoid muscle. The mass of vessels measured approximately 20 mm in diameter and consisted of multiple distended veins with increased caliber of the adjacent erector spinae, posterior neck veins, and the left jugular vein. The arteriovenous malformation arose from the internal maxillary artery and was situated in the left infratemporal fossa and pterygopalatine region. There was demonstrable shunting of blood with distension of the venous structures in the neck.

She was referred to an interventional radiologist and subsequently underwent embolization of the arteriovenous malformation under radiological guidance. A left carotid angiography demonstrated a large (approximately 2 to 3 cm in diameter) fistula arising from the internal maxillary artery communicating with the pterygoid venous plexus (Figs. 2 and 3).

The in-flowing vessel was localized with a 5 French guiding catheter, and several small platinum coils were placed to occlude the vessel. Angiography at the end of the procedure showed complete occlusion of the fistula (Figs. 4 and 5).

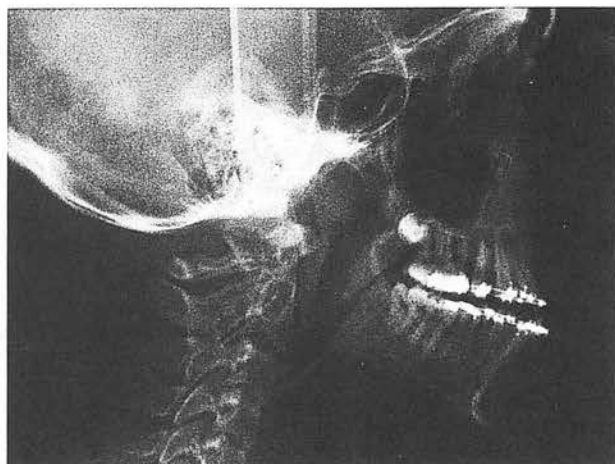


FIGURE 1 Preoperative lateral cephalogram.

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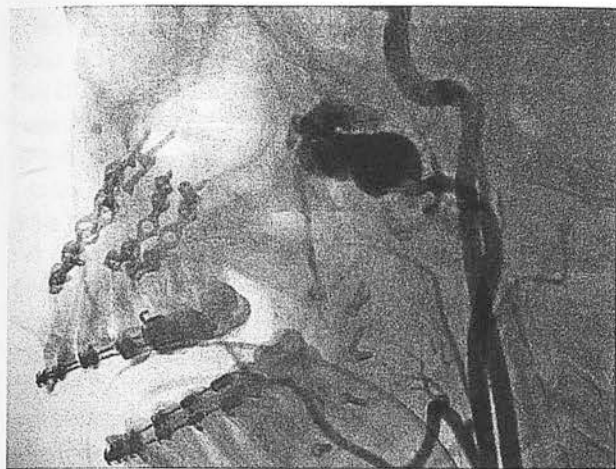


FIGURE 2 Left carotid angiogram, lateral view.

Postoperatively, the patient had an immediate and complete resolution of her symptoms. Similarly, at 6 months, she remains asymptomatic.

DISCUSSION

The Le Fort I osteotomy is associated with both minor and major complications. Kramer et al. (2004) analyzed the occurrence of complications following such surgery according to the presence of anatomical irregularities (e.g., in patients with cleft lip and palate, craniofacial dysplasias, or vascular malformations). They reported an overall complication rate of 6.4% in all patients following Le Fort I osteotomy. However, this was considerably higher in the group of patients with anatomical irregularities (25.2%) compared with the group with no anatomical irregularities (3.9%).

Major complications following Le Fort I osteotomy are fortunately very rare, but cases of arteriovenous fistulae (Albernaz and Tomsick, 1995), brain abscess (Baker et al., 1999), and even blindness (Lo et al., 2002) have been reported. Ar-

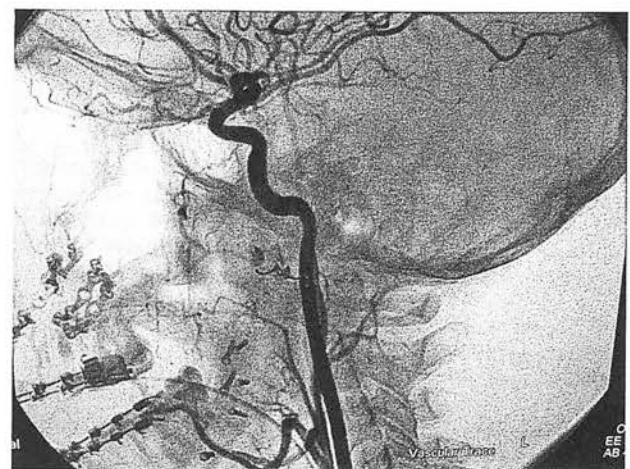


FIGURE 4 Postoperative angiogram, lateral view.

teriovenous fistulae have been known to occur elsewhere in head and neck surgery, for example, following rhinoplasty or tooth extraction (Albernaz and Tomsick, 1995). They are thought to arise as a result of a laceration of an artery with simultaneous laceration of a vein. The resulting hematoma is then subjected to endothelial proliferation, and endothelial-lined channels are formed between the artery and vein (Lanigan et al., 1991). Blood is then shunted directly from the high-flow arterial system to the low-pressure venous system, and lack of resistance in the fistula leads to preferential flow through the fistula. This often results in a bruit or thrill and in this case manifested itself as a pulsatile tinnitus because of its close proximity to the ear.

The internal maxillary artery is at particular risk because of its close relationship to the pterygomaxillary junction in the pterygopalatine fossa. During pterygomaxillary separation with the osteotome, the pterygoid plate may fracture in an unpredictable fashion and even extend to the skull base. This atypical fracture pattern may be responsible for the tears in the internal maxillary artery and pterygoid veins, which then results in an arteriovenous fistula.

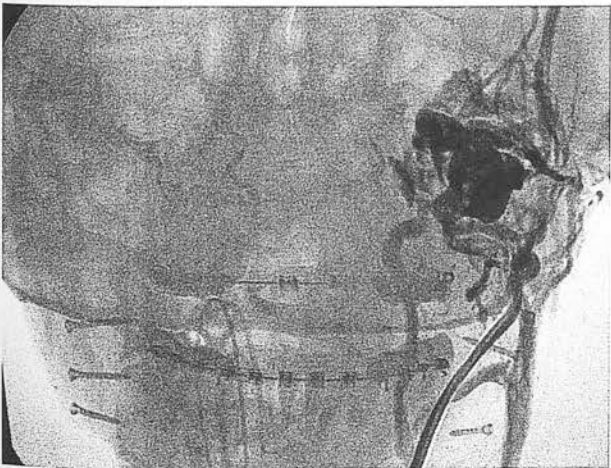


FIGURE 3 Left carotid angiogram, posterior-anterior view.

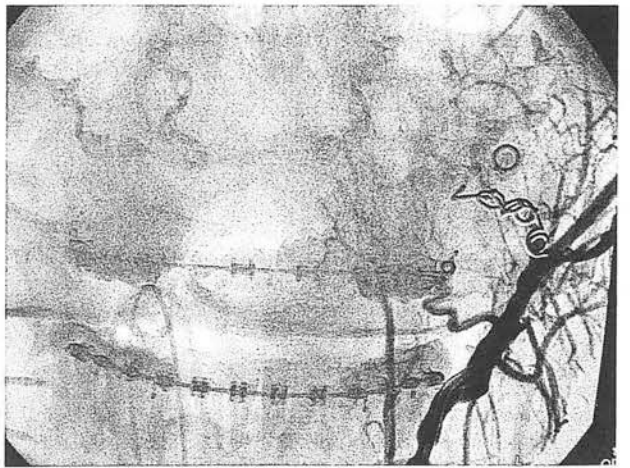


FIGURE 5 Postoperative angiogram, posterior-anterior view.

Previously, Rogers et al. (1995) described cases of posttraumatic aneurysm of the maxillary artery, one following a Le Fort III facial type fracture and the other following a Le Fort I maxillary advancement in a patient with a bilateral cleft palate and extreme midfacial hypoplasia. Lo et al. (2002) reported 2 cases of blindness following Le Fort I osteotomies on patients with cleft lip and palate. Patients with cleft lip and palates often have hypoplastic and retruded maxillae, and in addition, the palate and pterygoid area may be sclerotic and scarred as a result of previous palatal surgery. This anomalous anatomy in this group of patients may account for the unpredictable fracture pattern in the pterygomaxillary region when performing the Le Fort I osteotomy and the subsequent damage to the vessels in that region.

CONCLUSION

The complication of arteriovenous malformations is uncommon following Le Fort I osteotomy, but those with cleft palate may be at increased risk. It can be treated effectively with

radiological embolization, which is low risk and associated with minimal morbidity.

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Spinal Anomalies in Goldenhar Syndrome

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Objective: Goldenhar syndrome consists of the triad of craniofacial microsomia, ocular dermoid cysts, and spinal anomalies. The exact nature of the spinal anomalies remains poorly defined in the existing craniofacial literature, possibly due to these anomalies being managed by orthopedic surgeons rather than by craniofacial surgeons. The aim of this study was to clarify the nature and extent of these spinal anomalies.

Method: Review of case notes of patients who had their diagnosis confirmed following review by a clinical geneticist and in conjunction with radiographs (supplemented by three-dimensional computed tomographic [CT] scans where available).

Results: Seven patients fulfilled the entry criteria and had material available for study. A wide range of anomalies was present, including butterfly vertebrae; hemivertebrae which produced secondary scoliosis; kyphosis; and rib anomalies. Anomalies occurred at all levels within the spine.

Conclusion: The possibility of spinal anomalies at all levels of the spine should be considered by those treating cases of Goldenhar syndrome, because these anomalies can not be predicted from the severity of the facial malformation.

KEY WORDS: *fusion, Goldenhar, spine*

Goldenhar syndrome was first recognized in 1952 (Goldenhar, 1952). The syndrome consists of the triad of (usually unilateral) maldevelopment of the first and second branchial arches, ocular dermoids, and vertebral anomalies. All three signs have a variable presentation and are considered part of a spectrum of auriculo-vertebral anomalies (Figuerola and Friede, 1985).

The spinal anomalies have been poorly defined, partly due to the rarity of the condition, which has led previous researchers to include cases of craniofacial microsomia, as well as Goldenhar syndrome (Figuerola and Friede, 1985; Gibson et al., 1996), or to concentrate just on the cervical spine (Gossain et al., 1994). In addition, the clinical management of most cases is dominated by specialists whose primary interest is in facial reconstruction, so few patients undergo full clinical and radiological evaluation of the spine.

This report outlines an evaluation of these spinal anomalies in those patients with Goldenhar syndrome in whom the diagnosis of Goldenhar syndrome had been confirmed following assessment by a clinical geneticist.

No financial support has been provided to support this study.

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METHOD

Patients were identified from the departmental database. Case notes were reviewed and only those patients who were formally assessed by clinical geneticists and who had their diagnoses confirmed were included in the study. Other inclusion criteria included spinal radiographs (which were supplemented by computed tomographic [CT] studies, particularly of the cervical spine) and chest radiographs.

RESULTS

Fifteen cases of Goldenhar syndrome were identified from the database. However, only seven cases had full spine radiographs which could be retrieved for study. These consisted of two women and five men. These patients had undergone full spinal examination AP and lateral on at least one occasion, two also had undergone repeat examinations, and two more patients had additional AP and lateral cervical spine studies. Lateral cephalograms were not used, because it is recognized that they may be inadequate to visualize the lower cervical spine (Anderson et al., 1997a).

The radiographs displayed congenital anomalies throughout the spine (Table 1). The congenital anomalies commonly consisted of butterfly vertebrae, hemivertebrae, supplemental vertebrae, and rib anomalies, which are shown in Table 2 (Figs. 1 through 3).

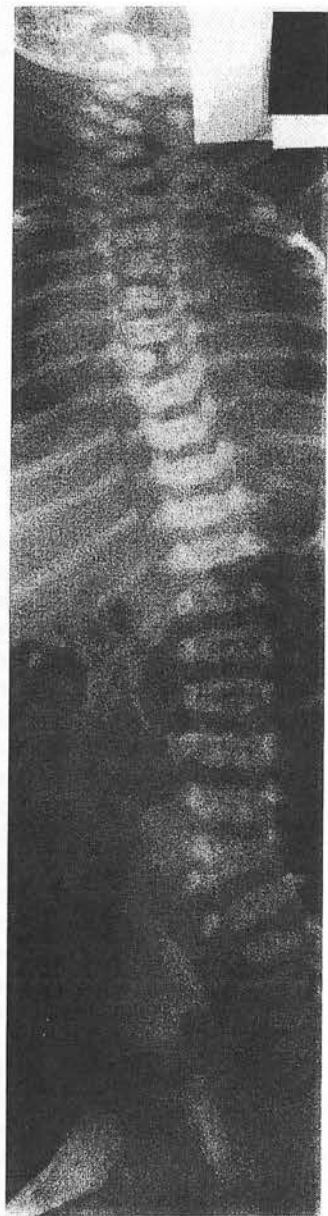


FIGURE 1 Case 1: AP whole spine, age 2 months with anomalies in cervical, thoracic, and lumbar regions.

DISCUSSION

The combination of eye, ear, and spinal anomalies was reported by Goldenhar (1952), and subsequently, this combination of malformations became recognized as an eponymous syndrome (Goldenhar, 1952). However, this syndrome represents just the severe end of the spectrum of all auriculo-vertebral anomalies (Figueroa and Friede, 1985; Goret-Nicaise et al., 1997). Its etiology remains uncertain; many cases are sporadic, but familial cases have been identified also (Rollnick and Kaye, 1983). More recently, it has been suggested that

TABLE 1 The Regions of Congenital Anomalies

Case No.	Sex	Age at Study	Study	Regions at Which Spinal Anomalies Identified	Rib Anomalies
1	m	2/12	full spine	cervical, thoracic, lumbar	no
2	m	8	full spine	cervical, thoracic	yes
3	f	14/12, 10 y	full spine, c spine	cervical, thoracic	yes
4	f	3/12, 5	full spine × 2	cervical, thoracic	yes
5	m	3, 5	full spine × 2	cervical, thoracic	yes
6	m	10	full spine, c spine	cervical, thoracic	yes
7	m	12/12	full spine	cervical, thoracic	yes

there is a focal disturbance in chondrogenesis (Goret-Nicaise et al., 1997), although it has been postulated that the etiology is due to an error in embryological development of ectoderm (Lam, 2000). Comprehensive understanding of the embryological events that result in the Goldenhar syndrome is hindered when the extent of one of the triad of anomalies (the extent of the spinal anomalies) remains unclear in many cases. Although many individuals with spinal anomalies are asymptomatic, it has been recorded that these anomalies may become clinically significant and may produce spinal instability requiring spinal fusion (Healy et al., 2002).

The results of this study demonstrate that a range of congenital anomalies can occur at all levels of the spinal column. These commonly consist of butterfly vertebrae and hemiver-

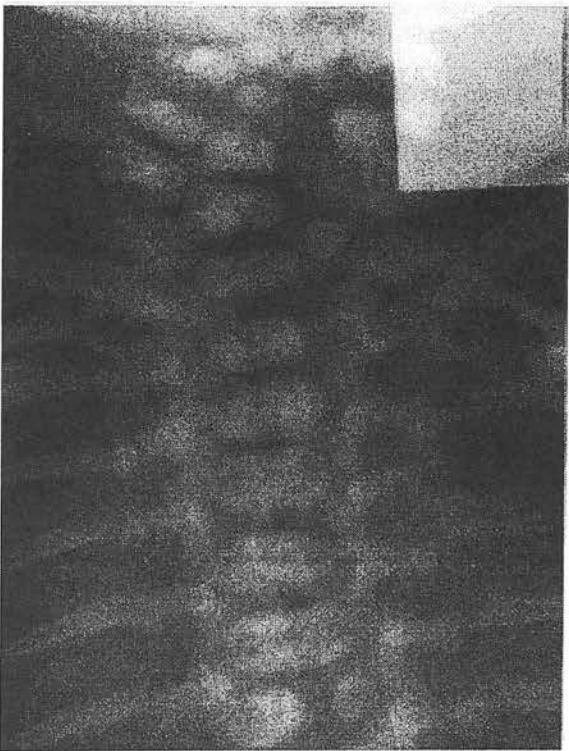


FIGURE 2 Case 2: AP spine block fusion C6/C7/T1. Note also the scoliosis.

TABLE 2 The Types of Anomalies and Facial Deformity

Case No.	Sex	Cervical	Thoracic	Lumbar	Ribs	SAT Score
1	m	B.V. C6*	fusion defect T6	H.V. L5, fusion L5/S1, scoliosis	normal	S3, A3, T3
2	m	block fusion C6/C7/T1, scoliosis	B.V. T4, scoliosis	normal	fusion left 3rd/4th	S5, A3, T3
3	f	fusion C3/C4, scoliosis, hypoplastic odontoid	fusion T2/T3/T4, H.V. T6, scoliosis	bilateral sacralization S5, scoliosis	fusion left 1st/2nd	S5, A2, T3
4	f	assimilation of atlas to base of skull, fusion C5/C6, scoliosis	H.V. T8, scoliosis	normal	aplasia: 2 left ribs, 1 right rib	S1, A2, T1
5	m	assimilation of atlas to base of skull, scoliosis	H.V. T4	normal	cervical ribs	S1, A1, T1
6	m	assimilation of atlas to base of skull, fusion C3/C4	normal	normal	hypoplasia: left 1st/2nd, right cervical ribs	S5, A3, T3
7	m	B.V. C4, scoliosis	H.V. T6	normal	aplasia: left 12th rib, left cervical rib	S2, A1, T1

* B.V. = butterfly vertebra; H.V. = hemivertebra.

tebrae, which both have been reported in Craniosynostosis syndromes also (Anderson et al., 1997a,b). This study also confirms previous studies that anomalies in the cervical region commonly occur (Darling et al., 1984; Figueroa and Friede, 1985; Gibson et al., 1996). However, although anomalies of the thoracic spine have been noted (Avon and Shively, 1983), we have found in our series, where full radiological evaluation of the spine has been undertaken, that these anomalies coexist with those in the cervical region.

We speculate that our finding probably is due in part to the rarity of the Goldenhar syndrome and in part to the fact that most cases are managed by clinicians whose main concern is the function and cosmetic appearance of the face. (Within our own series, only half of the patients who met the inclusion criteria by diagnosis had full spinal radiographs available, these cases being largely historical and the patients no longer being treated). The significance is that the absence of any symptoms and an unremarkable clinical appearance may disguise significant anomalies below the cervical spine. So far, none of our patients have required spinal surgery (unlike those in a previous report of Healy et al. 2002), but five of the seven cases remain under regular orthopedic review.

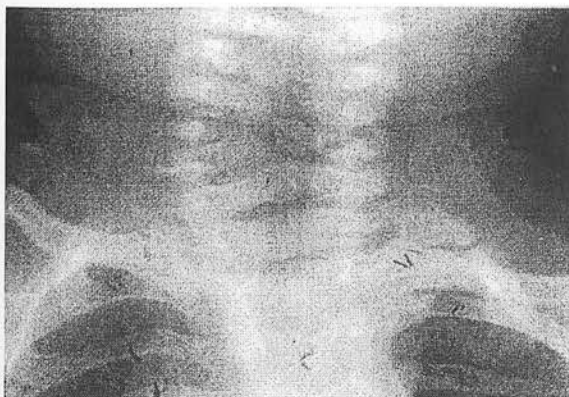


FIGURE 3 Case 7: AP Spine C4 butterfly vertebra, cervical rib left side.

To investigate whether the severity or site of the spinal anomalies could be predicted from the severity of the facial anomalies, we compared the severity of facial involvement using the SAT score, which measures skeletal, soft tissue, and auricle deformities (David et al., 1987) with the spinal anomalies. The SAT scores for each patient are shown in Table 2. A wide range of severity of facial anomalies is present in these cases, and no obvious association could be demonstrated. This suggests that clinical assessment of the face is a poor guide for predicting the extent or level of spinal anomalies.

In conclusion, we have found a wide range of spinal and rib anomalies in cases of Goldenhar syndrome. The anomalies occurred with almost equal incidence in the cervical and thoracic spine of those who had whole spine assessment. The lumbar spine was less commonly affected. Because we could find no correlation with the severity of facial deformity, we suggest that all clinicians be aware of the possible existence of spinal anomalies, which may be in the thoracic region, and which may not be readily clinically apparent.

Finally, it is interesting to speculate that because Goldenhar syndrome is only part of the spectrum of all auriculo-vertebral anomalies, the further study of the spine of all those with auriculo-vertebral anomalies, including the less severely affected craniofacial microsomia, would help clarify the relationship between these conditions.

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CASE REPORTS

Modified costochondral graft osteotomy in hemifacial microsomia

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KEYWORDS

Hemifacial microsomia;
Orthognathic surgery; Rib
graft

Summary Hemifacial microsomia is the second most common facial clefting condition after cleft lip and palate. The deformity affects the skeleton and soft tissues in the temporal region of the affected side, although the degree of involvement is markedly variable.

We describe a modification of surgical technique in a skeletally mature case who had previously undergone mandibular reconstruction with a costochondral graft.

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Reconstruction of the mandibular ramus and temporomandibular joint in hemifacial microsomia in early childhood using a costochondral graft is well established¹ and we wish to report a modification in technique that was required when an adult patient subsequently underwent bi-maxillary surgery.

The patient originally had a Pruzansky grade 3 mandible on the left side, reconstructed with a costochondral graft when aged eight years which had overgrown, it being well recognised that graft growth can be difficult to predict.² At the age of 16 years this resulted in a significant deviation of the mandible to the nonaffected side and to the development of a cross bite. Corrective surgery consisted of maxillary centralisation and advancement, with a vertical subsigmoid osteotomy on the normal side of the mandible and a modified inverted 'L' on the reconstructed side.³

At the time of surgery the maxillary osteotomy and the right mandibular osteotomy proceeded in the classical manner. However, the osteotomy on the left (reconstructed) side was modified due to the significant graft overgrowth, thus not disturbing the satisfactory functioning of the modified temporomandibular joint or compromising the blood supply to the rib graft. The position of the transverse cut of the modified osteotomy could be positioned more inferiorly because of the absence of the inferior alveolar foramen, and this is shown on the nylon model (Fig. 1). This was achieved via an external approach utilising the old scar from the rib graft insertion. The pre-determined occlusal position was easily obtained and the mandible was fixed using iliac crest bone graft and lag screws. The post-operative recovery was uneventful and the position has been maintained.

We would recommend that surgeons undertaking the management of hemifacial microsomia may wish to consider this surgical modification if faced with a similar clinical situation.

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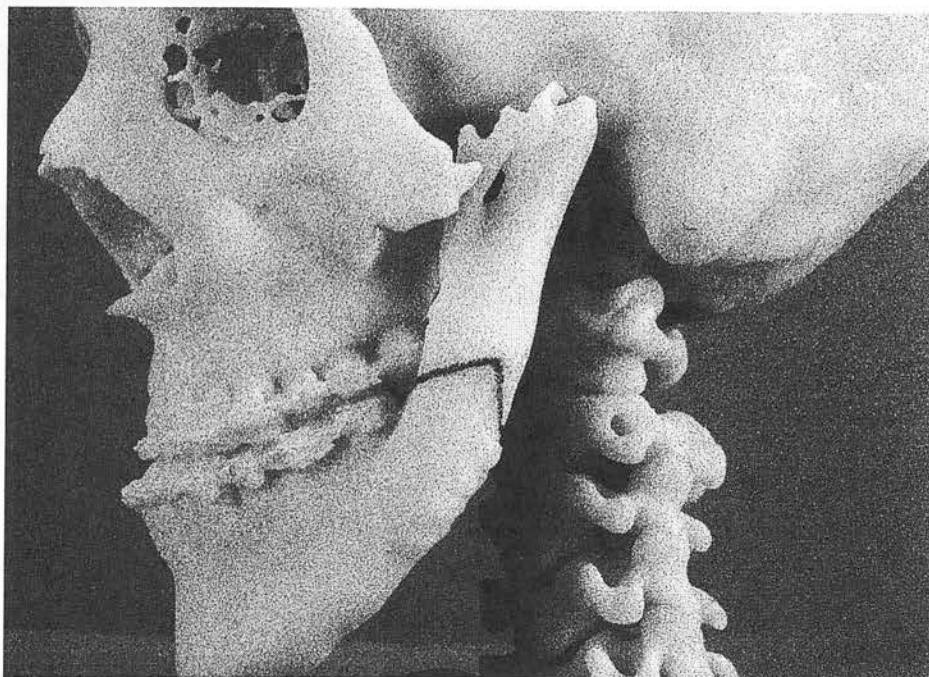


Fig. 1 The customised nylon model of the patient, with the position of the modified osteotomy marked on the reconstructed mandible. The outline of the fixation plate used at the time of graft placement can be seen along the lower border.

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Sentinel node biopsy in patients with in-transit recurrence of malignant melanoma

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KEYWORDS

Melanoma; Sentinel node; In-transit metastasis

Summary Sentinel node biopsy (SNB) is now widely used for accurate staging of patients with clinical stage I or II malignant melanoma. We describe the use of SNB in five patients with in-transit recurrence (stage IIIB) and demonstrate that it provides accurate staging of the lymph nodes in this group of patients.

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Van der Woude syndrome: dentofacial features and implications for clinical practice

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ABSTRACT

Background: Van der Woude syndrome (VWS) is the most common clefting syndrome in humans. It is characterized by the association of congenital lower lip fistulae with cleft lip and/or cleft palate. VWS individuals have a high prevalence of hypodontia. Although caused by a single gene mutation, VWS has variable phenotypic expression. This study aimed to describe the range of clinical presentations in 22 individuals with VWS to facilitate its diagnosis.

Methods: A retrospective study of 22 patients with a diagnosis of VWS was undertaken at the Australian Craniofacial Unit (ACFU) in Adelaide. Three extended families with affected members were included in the study cohort.

Results: The overall prevalence of lip pits in this study cohort was 86%. Cleft phenotypes included bilateral cleft lip and palate (32%); unilateral cleft lip and palate (32%); submucous cleft palate (23%); and isolated cleft hard and soft palate (9%). Missing permanent teeth were reported in 86% of affected individuals.

Conclusions: Submucous cleft palate in VWS may go undiagnosed if the lower lip pits are not detected. Associated hypodontia and resultant malocclusions will also require management by a dental team.

Keywords: Cleft, diagnosis, genetic counselling, hypodontia, lip pits.

Abbreviations and acronyms: ACFU = Australian Craniofacial Unit; CL = cleft lip; CLP = cleft lip and palate; ICP = isolated cleft palate; IRF6 = interferon regulatory factor six; MIH = molar incisor hypomineralization; SMCP = submucous cleft palate; VWS = Van der Woude syndrome.

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INTRODUCTION

Van der Woude syndrome (VWS; OMIM #119300) is characterized by the association of congenital lower lip fistulae with cleft lip and/or cleft palate.¹ It is inherited as an autosomal dominant clefting syndrome that shows high penetrance but variable expressivity amongst carriers. The eponymously named VWS was first described by Anne Van der Woude who detailed the association between congenital pits of the lower lips and cleft lip and palate in 1954.² She determined that the condition, later to be known as Van der Woude syndrome, was a complex inherited by a single gene that elicited variable expressivity ranging from lower lip pits with cleft lip and palate to no visible abnormalities. The reported incidence of Van der Woude syndrome varies in the literature from 1:100 000 to 1:40 000 births.^{3–6} VWS is present in approximately 2% of facial cleft patients and is often referred to in the literature as the most common clefting syndrome.⁷

VWS has been reported to show near complete penetrance and equal gender distribution.^{4,7}

The defining characteristics of VWS are the congenital orofacial anomalies which include distinctive congenital lower lip pits.^{7,8} These are the principal manifestations of VWS and they occur in 88% of affected individuals. In 64% of cases they have been reported to be the only defect detected.⁴ Other cardinal signs of VWS include hypodontia in addition to orofacial clefting.^{4,9}

Almost all cases of VWS have shown linkage to a region at chromosome 1q32–p41, known as VWS locus 1. However, some affected individuals have instead shown linkage to a second chromosomal locus (VWS locus 2), which is located at 1p34.¹⁰ More recently, a gene responsible for VWS has been identified. A mutation affecting the gene that encodes for interferon regulatory factor six (IRF6), which is located at the VWS critical region of 1q32–p41, underlies the majority of VWS cases.¹⁰ It is of great interest that a

monogenic mutation can cause such a wide range of effects. Modifier loci have also been proposed to account for the highly variable expression of VWS.¹¹

Diagnosis of VWS can be made clinically, based on the presence of congenital lip pits and other orofacial anomalies, which may occur together or exclusively. Most of the developmental anomalies are congenital and hence clinical diagnosis may occur soon after birth for the majority of cases. There is a varying degree of expressivity that must be accounted for when diagnosing patients. Genetic counselling is also vital in the definitive diagnosis and management of affected individuals.

The distribution of clinical phenotypes of VWS described in the literature varies greatly and for those cases that are not diagnosed around birth, dentists will be on the frontline in diagnosing the condition. Therefore, it is important that they have some understanding of the nature and extent of phenotypic variation in affected individuals. The aim of this study was to describe the range of clinical presentations in 22 individuals with VWS with a view to facilitating diagnosis of the syndrome.

MATERIALS AND METHODS

Study sample

This was a retrospective study of 22 patients with a diagnosis of Van der Woude syndrome, undertaken at the Australian Craniofacial Unit (ACFU) in the Women's and Children's Hospital in Adelaide, Australia. Management of cleft lip and palate children has been undertaken for over 30 years at the ACFU. Both South Australians and patients from other states have been treated by a multidisciplinary team and the ACFU has an extensive database of VWS patients. There were three extended families with affected members included in the study cohort. Ethical consent (WCH: 209A) was obtained to clinically examine these affected individuals, as well as evaluate their medical case notes.

The frequency of VWS amongst cleft patients at the ACFU and in the general Australian population was calculated. The mode of inheritance and penetrance rate of VWS were also determined. Penetrance rate was determined from the number of VWS patients with a clinical manifestation divided by the total numbers of carriers and patients expressing VWS phenotypes. Gene carriers without clinical phenotype were identified from pedigree analysis.

Genetics

Some case notes contained reports of genetic counselling that confirmed the presence of the syndrome in the

affected individual or in one of their affected family members. Genetic testing for the mutation responsible for VWS is not yet available in Australia.

Clinical manifestations

Clinical manifestations were determined by medical case-note review and verified against archived clinical photographs. Oral examination of some patients was carried out by the authors and compared with documented findings. The presence, type and location of lip pits were recorded. The presence or absence of cleft lip and/or cleft palate was recorded. Information on affected family members, who could not be contacted or were deceased, was obtained from interviews with other relatives.

Dental anomalies

The prevalence of hypodontia and/or presence of any dental malocclusion was determined by clinical examination and review of relevant radiographs obtained at the ACFU.

RESULTS

There was a total of 22 VWS affected patients on the ACFU database, including the members of three extended families. The prevalence of VWS among facial cleft patients was 1.5% (10 male and 12 female patients with VWS among a total of 1482 facial cleft patients on the ACFU database). The prevalence of VWS in the general Australian population is estimated to be 1 in 70 000. The gender distribution of affected individuals was almost even (45% male and 55% female).

There were 19 patients with lower lip pits or sinuses. Of those, 17 had bilateral symmetric lip pits. There was one report of asymmetry of the lip pits with one pit replaced by a nodule. There was also one report of a unilateral shallow lip pit. The overall prevalence of lip pits as a VWS phenotype in this study cohort was 86%. The lip pits were removed in early childhood prior to development of symptoms.

Cleft phenotypes displayed in ACFU patients included bifid uvula, submucous cleft palate, isolated cleft palate, incomplete and complete unilateral or bilateral cleft lip and palate (Table 1). Seven patients (32%) had

Table 1. Cleft phenotypes presenting in ACFU patients

Cleft phenotype	No. of type/total (%)
Bilateral cleft lip and palate	7/22 (32)
Unilateral cleft lip and palate	7/22 (32)
Isolated cleft palate	2/22 (9)
Submucous cleft palate	6/22 (27)
Cleft lip	0/22 (0)

bilateral cleft lip and palate, seven patients (32%) had unilateral cleft lip and palate, five patients (23%) had submucous cleft palate, and one more patient displayed a bifid uvula, which is a microform of the submucous clefting phenotype. Two patients (9%) had isolated cleft hard and soft palate. None of the patients had a cleft lip anomaly only.

Other anomalies that occurred in three of the VWS affected individuals included molar incisor hypomineralization (MIH), patent ductus arteriosus, and impaired neuropsychological development.

There were many cases of hypodontia in this study cohort. Nineteen of the 22 (86%) VWS affected individuals had reports of missing permanent teeth. The most common missing teeth were the permanent maxillary lateral incisors associated with the respective unilateral or bilateral clefting phenotype. Six of the 19 patients who were reported to have hypodontia had missing second premolars. All four different clefting phenotypes – unilateral cleft lip and palate, bilateral cleft lip and palate, isolated cleft palate and submucous cleft palate – were found to occur with hypodontia.

Analysis of family trees of the three extended families with affected members revealed an autosomal dominant mode of inheritance of VWS. VWS family 1 was shown to have an affected member in each generation as well as close to 100% penetrance (Fig 1). Family tree diagrams of the two other extended families in the study population were not included due to inadequate information on some affected individuals.

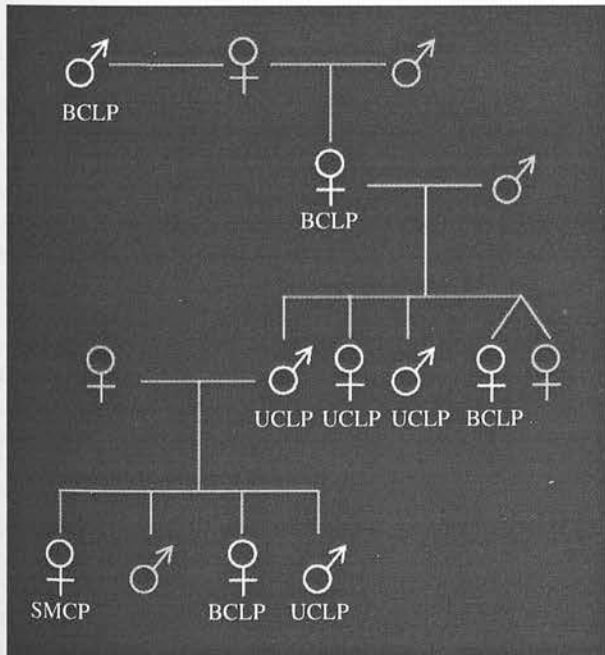


Fig 1. Family tree of Van der Woude family 1.

DISCUSSION

Van der Woude syndrome has been determined to display an autosomal dominant mode of inheritance. This is reflected in the extended families who had multiple VWS affected members in each generation, as well as male-to-male transmission. The reported penetrance rate of VWS ranges from 80% to 100%. Our study population exhibited close to 100% penetrance, which agreed with the results of previous VWS epidemiological studies.^{4,7}

The prevalence of VWS among cleft populations has been reported in the literature to range from 0.37% to 6%.¹² A prevalence of 1.5% was noted amongst cleft patients from the ACFU. From the results of this study, the prevalence of VWS in the Australian population can be estimated to be around 1 in 70 000. This is similar to what has been previously reported: 1 in 60 000 to 1 in 100 000 live births for Caucasian populations.^{4,7} Therefore, it is likely that there are approximately 300 individuals affected by VWS living in Australia at the present time. It is reasonable to suggest that VWS individuals in Australia do not have an increased rate of mortality due to the availability of adequate health care to treat the congenital defects. There was no discernible difference in gender distribution of affected individuals, which was consistent with the results of previous studies.^{7,13}

Patients in this study exhibited a full range of cleft phenotypic expression. Cleft types that were displayed included bifid uvula, submucous cleft palate, isolated cleft palate, and incomplete and complete unilateral or bilateral cleft lip and palate. This highlights the high degree of phenotypic variation in VWS. The cleft-type distribution differs among different studies (Table 2). According to previous studies, approximately two-thirds of cleft patients have cleft lip and palate, and the remaining one-third have cleft palate.¹¹ In this series, approximately 64% displayed cleft lip and palate and approximately 36% had cleft palate only (including patients with submucous cleft palate only).

In this study, no VWS patients were affected with cleft lip only (without cleft palate). This finding is similar to those of previous studies that have reported no cases or very low prevalence of the cleft lip only clinical phenotype.^{5,7,13} However, a study by Onofre *et al.*¹⁴ showed that over 16% of a South American VWS sample had a cleft lip anomaly.

In this series there was a high proportion of VWS patients who had submucous cleft palate. Twenty-seven per cent of the VWS cases had submucous cleft palate compared with 13% in a study by Shprintzen *et al.*⁷ and 8% in a study by Rintala and Ranta.⁵ All six cases of submucous cleft palate in this study occurred with bilateral lower lip pits or sinuses. This highlights the importance of diagnosis of VWS from the congenital

Table 2. Comparison of distribution of cleft phenotypes between this study and other studies

	This study	Huang <i>et al.</i> (2007)	Martelli-Junior <i>et al.</i> (2007)	Onofre <i>et al.</i> (1997)	Rintala and Ranta (1981)	Shprintzen <i>et al.</i> (1980)
CLP	64%	83%	80%	67%	37%	30%
ICP	9%	17%	5%	17%	43%	21%
SMCP	27%	0%	0%	0%	8%	13%
CL	0%	0%	10%	16%	0%	4%
No cleft	0%	0%	5%	0%	13%	34%
Total	22	64	20	133	79	57
Country	Australia	Taiwan	Brazil	Brazil	Finland	USA

CLP = cleft lip and palate; ICP = isolated cleft palate; SMCP = submucous cleft palate; CL = cleft lip.

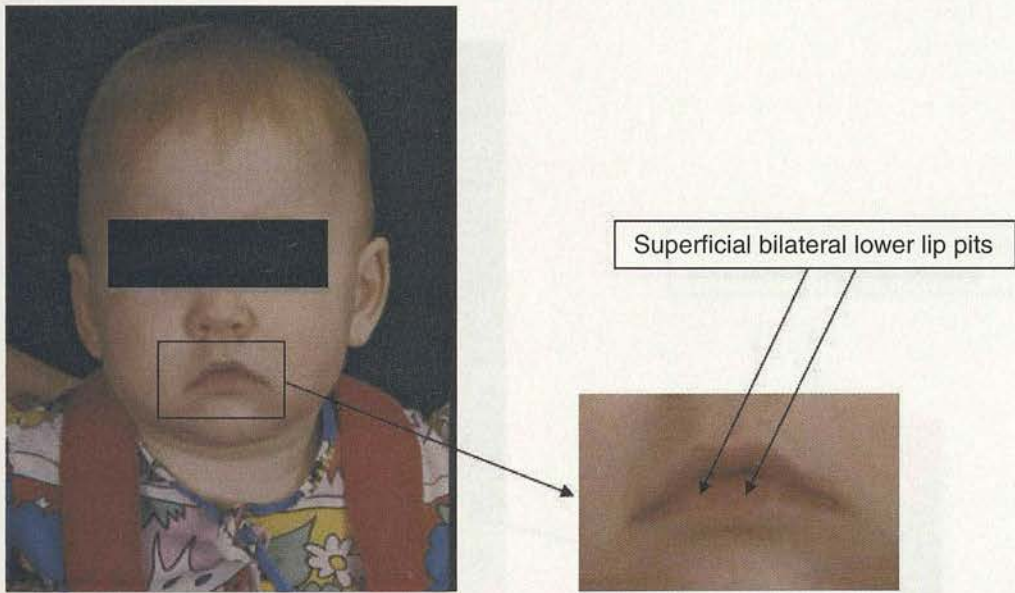


Fig 2. Affected female with very superficial congenital bilateral lower lip pits that were only detectable on probing. She also had submucous cleft palate. This highlights the importance and difficulty of diagnosis of VWS from lip pits alone.

lower lip pits or sinuses. Figure 2 shows a VWS based on female displaying characteristic bilateral lower lip pits. However, she also has a submucous cleft palate. The submucous cleft palate could not be diagnosed from an extra-oral examination alone and, hence, the presence of the lip pits should alert the physician or dentist to the possibility of a submucous cleft palate in this patient. If lip pits are not detected and VWS is not diagnosed, then a submucous cleft palate that may have occurred with the lip pits will most likely go undiagnosed until later in childhood when the effects of the submucous cleft palate become evident (e.g., hearing loss).

Submucous cleft palate is clinically characterized by Calnan's triad.¹⁵ The triad of intra-oral features are bifid uvula, notching of the hard palate and lucency of the mucosa at the midline of the palate (Fig 3). Although the overlying mucosa of the cleft is intact, the underlying muscles have lost their correct attachments and are incorrectly aligned (similar to a cleft palate). Not all these features need to be present to



Fig 3. Affected male with submucous cleft palate. The soft palate cleft and bifid uvula are visible in this photograph. Also, note the translucent mucosal line along the midline of the hard palate which indicates a submucous cleft of the hard palate.

diagnose this condition.¹⁶ A submucous cleft palate can be easily mistaken at birth for a normal palate. Hence, dentists must be aware of the characteristic clinical

features of this condition as they are often at the frontline of its diagnosis.

The main problems associated with submucous cleft palate are feeding difficulties at birth, impaired speech development, middle ear pathology and facial growth problems.¹⁵ However, only 10% of cases are symptomatic.¹⁷ The feeding problems during infancy often occur together with nasal regurgitation.¹⁵ Newborn infants should be examined intra-orally both visually and by palpation with a finger. Any feeding difficulties without an obvious cause should alert the physician or dentist to the possibility of submucous cleft palate.¹⁵

The definitive diagnosis of submucous cleft palate may be delayed until speech problems are identified, which is reported to be at two and half years of age when speech can be assessed accurately.¹⁷ Studies have shown that hypernasal speech and loss of labial sounds as a result of velopharyngeal incompetency are commonly associated with submucous cleft palate. Nasality is particularly noticeable on high vowels such as ee/i/ and oo/u/.¹⁸ Submucous cleft palate has also been shown to have a high frequency of association with middle ear disease (chronic otitis media) and hearing loss, which requires surgical intervention and insertion

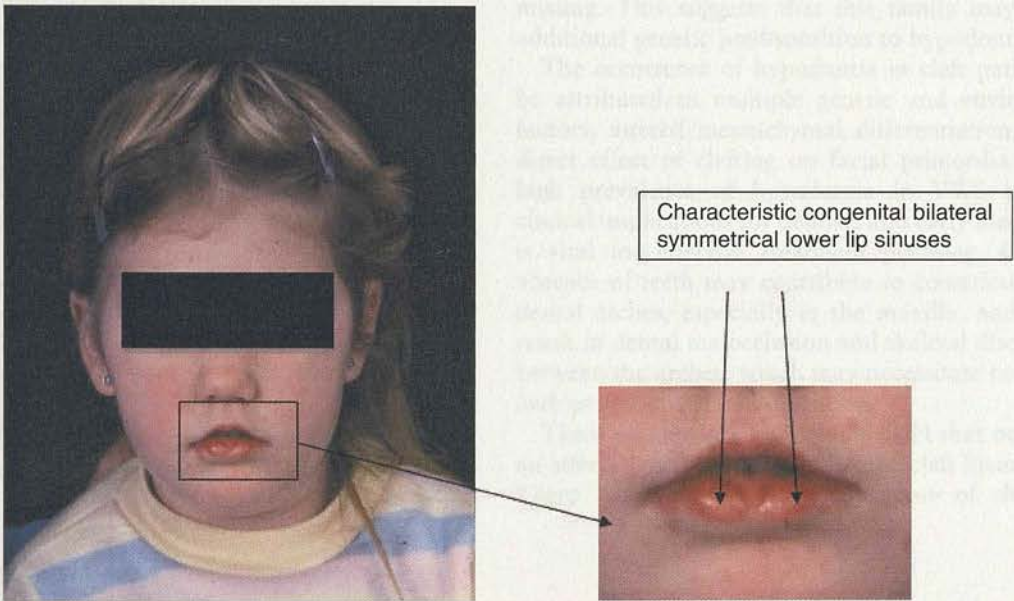


Fig 4. Affected female with characteristic bilateral lower lip sinuses. She also has submucous cleft palate.

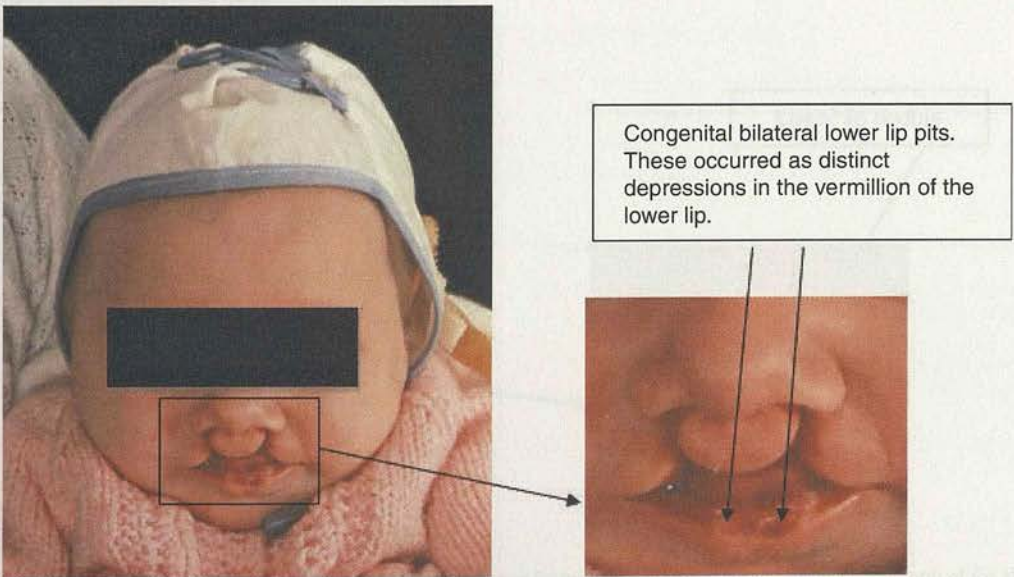


Fig 5. Affected female with characteristic bilateral lower lip sinuses and a bilateral cleft lip and palate.

of ventilation tubes (ear grommets) to prevent infection and damage to the delicate middle ear structures.¹⁹

The strong association between hypodontia and VWS has been established previously.²⁰ In this study, 60% of the study cohort were reported to have missing permanent teeth. This percentage may well have been higher if better dental records had been obtained from VWS family 1, but this was not possible. Twenty-seven per cent of this VWS cohort displayed missing second premolars. This is slightly higher than the 18% of cleft lip and palate patients who were reported by Shapira *et al.*²¹ to have missing second premolars. A study by Oberoi *et al.*²² also showed that congenital absence of the mandibular second premolars was more common in

patients with VWS than in matched cleft controls. Interestingly, in this study, the severity of the clefting phenotype did not correspond with the frequency of hypodontia as had been reported by Oberoi *et al.*²² In fact, an affected female with bifid uvula had the greatest number of missing adult teeth. Bifid uvula is considered to be a microform of clefting, hence the least severe clefting phenotype corresponded with the highest frequency of hypodontia. An affected male with a sub-mucous cleft of the hard palate from the same family had the next most severe expression of hypodontia with four missing adult teeth. All four affected individuals from this family were reported to have multiple teeth missing. This suggests that this family may have an additional genetic predisposition to hypodontia.

The occurrence of hypodontia in cleft patients may be attributed to multiple genetic and environmental factors, altered mesenchymal differentiation, and the direct effect of clefting on facial primordia.^{22,23} The high prevalence of hypodontia in VWS has direct clinical implications for dentists and early identification is vital for effective treatment planning. Congenital absence of teeth may contribute to constriction of the dental arches, especially in the maxilla, and this can result in dental malocclusion and skeletal discrepancies between the arches, which may necessitate orthodontic and/or orthopaedic intervention.

There was an isolated case of MIH that occurred in an affected male who had unilateral cleft lip and palate. There has been no previous report of this dental



Fig 6. Affected male with bilateral cleft lip and palate and characteristic congenital paramedian bilateral lower lip pits.

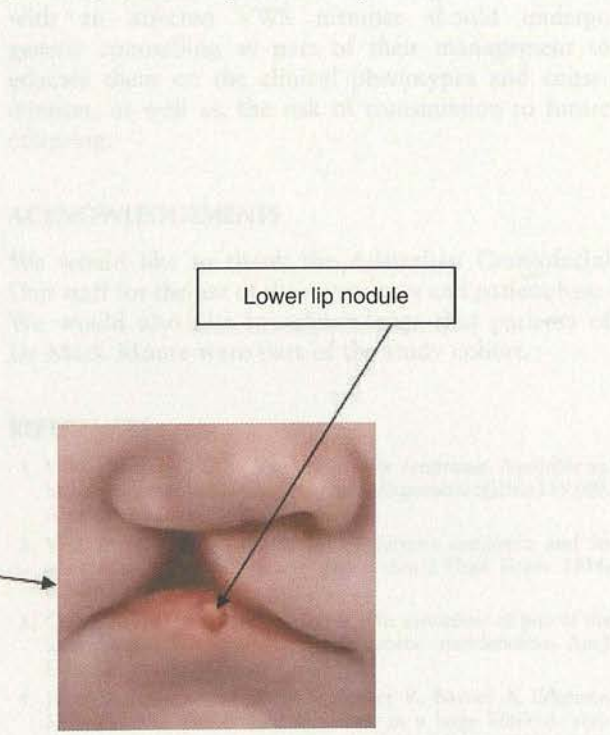
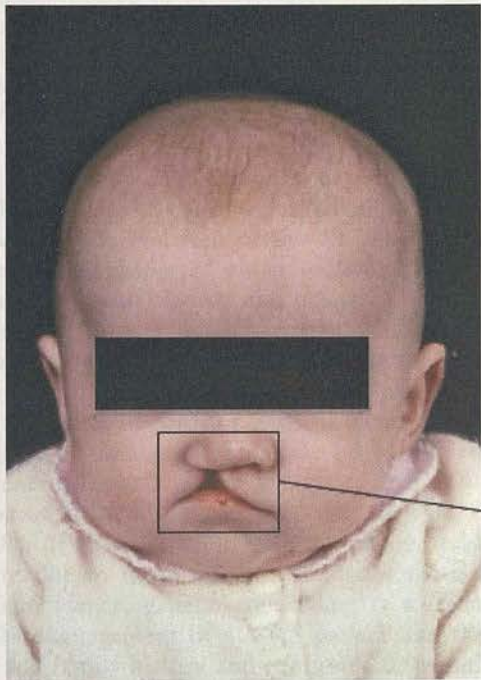


Fig 7. Affected female with unilateral cleft lip and palate and a unilateral lower lip nodule. This lower lip nodule is a microform of the characteristic lower lip pit.

anomaly occurring in a VWS affected individual in the literature. However, in this case MIH is likely to be an isolated independent anomaly and not an associated feature of VWS.

The overall prevalence of characteristic lower lip pits in this study cohort was 86%. Hence, detection of the lower lip pits is vital for the correct diagnosis of VWS in the vast majority of affected individuals. Dentists are on the frontline in diagnosing VWS. However, diagnosis of lip pits can be difficult because of several different types of lip pits that can occur in VWS (Figs 4–7). These include bilateral symmetric, bilateral asymmetric, unilateral (which are considered incomplete expressions of the trait), median and microforms.⁹ The microforms of the lip pits occur as conical elevations, transverse mucosal ridges, or openings with no depth.^{14,24}

In early childhood, the pits are often on the vermilion zone of the lower lip and are centered on top of conical elevations, but they progress to form simple depressions in adulthood, which can be asymptomatic.⁷ The sinus tract may enter the orbicularis oris muscle. Some fibres of this skeletal muscle have been found to be orientated in such a manner that, upon contraction, they induce a peristaltic ejection of mucous secretion. If the communicating ducts of the mucous acini are open then the fistulae may exude a mucin-like fluid.² Chronic discomfort (due to inflammation as a result of the salivary excretions and/or bacterial penetration) and poor aesthetics are common reasons cited for surgical removal of lip pits.⁹

The critical significance of lip pits is that their expression is reported to be associated with increased severity of the cleft lip and/or cleft palate. Studies reported in the literature have described the types of cleft associated with different types of lower lip pits.^{3,5,14,24} These investigations have shown that the bilateral, unilateral or mixed-type lip pits are more likely to occur with cleft lip with or without cleft palate. This finding was also reflected in the ACFU patients with VWS. Fourteen of the 19 patients who had lower lip pits displayed either unilateral cleft lip and palate or bilateral cleft lip and palate.

Diagnosis of lip pits in cleft patients has further implications for genetic counselling because the probability of a cleft patient who also has lip pits having offspring with cleft lip with or without cleft palate, is reported to be 10 times greater than the probability of a cleft patient without the lip pits.^{2,3,7,25} All VWS-affected parents should be cautioned that they carry a risk of 50% of having a child with a cleft lip and/or cleft palate due to its autosomal dominant mode of transmission. Hence, genetic counselling is a critical stage in the management of a VWS patient and their family. A full family history and confirmed differential diagnosis of VWS from other overlapping syndromes (e.g., popliteal pterygium syndrome)¹⁰ is needed before

counselling is given to family members. Information regarding the pattern of inheritance, the range of clinical manifestations, and the consequence of these phenotypes should be emphasized.

The sample size in this study is relatively small and may not be truly representative of VWS in Australia. However, this is the first study of VWS conducted in Australian families and it provides a foundation for further research based on larger numbers of families.

CONCLUSIONS

The highly variable phenotypic expression of VWS is demonstrated by the wide range of clefting phenotypes in this study cohort. The distribution of clefting phenotypes was not consistent with that reported in the literature, with a greater proportion of VWS individuals affected by submucous cleft palate. Under-diagnosis of VWS has been reported to be linked to lack of detection of the lower lip pits. As this study has shown, submucous cleft palate may go undetected if the lower lip pits are not detected and VWS is not diagnosed. Submucous cleft palate has been shown to result in feeding difficulties, speech and hearing impairment and facial growth problems. Hypodontia is also a significant phenotypic expression of VWS and will require management by a dental team. It is important that dentists appreciate the nature and extent of variation in clinical presentation of individuals with VWS and are proficient in its diagnosis. Families with an affected VWS member should undergo genetic counselling as part of their management to educate them on the clinical phenotypes and consequences, as well as, the risk of transmission to future offspring.

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Multidisciplinary Management of Opitz G BBB Syndrome

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Abstract: Opitz G BBB syndrome is a rare condition characterized by the 3 major anomalies of hypertelorism, cleft lip and palate, and hypospadias, although there may be other associated anomalies. The underlying genetic causes are complex and consist of both X-linked recessive and autosomal dominant forms of the disorder. Previously, there have been publications on the underlying genetics and case reports, but there have been few reports regarding the long-term outcome.

The aim in this study was to review the range of clinical presentation and evaluate outcomes of the multidisciplinary management of a cohort of patients with Opitz G BBB syndrome. In a 25-year period, 7 patients with Opitz G BBB syndrome were managed by the Australian Craniofacial Unit (ACFU), 5 male and 2 female. Most of the patients are now reaching skeletal maturity. Each one presented with a range of severity in the triad of hypertelorism, cleft lip and palate, and hypospadias anomalies. The males all exhibited the triad of anomalies, while the females both had hypertelorism, only 1 had isolated cleft palate, and neither had any genitourinary anomalies. Each patient underwent multidisciplinary assessment to make a treatment plan for staged management of different anomalies. Plan for surgical corrections of facial anomalies were performed according to the unit's protocol management of both hypertelorism and cleft lip and palate, but the presence of these coexisting anomalies required adjustment of the standard protocol of management of cleft lip and palate.

In conclusion, we recommend that patients with Opitz G BBB syndrome require careful evaluation, and management of the anomalies should be in a coordinated manner by a multidisciplinary team.

Key Words: Opitz syndrome, cleft lip and palate, hypertelorism, hypospadias, oculogenitourinary

(*Ann Plast Surg* 2005;55: 402–407)

Opitz G BBB syndrome is a congenital condition characterized by a variable spectrum of anomalies that include the triad of hypertelorism, cleft lip and palate, and hypospadias. Other anomalies include laryngo-esophageal abnormalities, imperforate anus, cardiac defects, and developmental delay. John Opitz originally described this condition as separate clinical entities, G and BBB syndromes: the names derived from the first letter of the family names of the patients described in the initial report.^{1,2} However, it is now widely recognized that the 2 original descriptions were variable presentations of a single entity.^{3,4} The incidence of this condition remains unknown and is difficult to determine because of the marked variability in clinical presentation. This is highlighted by a number of reports where retrospective clinical evaluation and genetic testing have revealed multiple mutation-positive, mildly affected relatives of probands.⁵

The condition is peculiar in that it is genetically heterogeneous, with both X-linked (Xp22.3) and autosomal (22q11.2) inheritance described.^{4,5} A contribution from both chromosomes may explain some of the clinical variability, although other unknown factors affect clinical presentation. Mutations in the MID 1 gene on the X chromosome have been found responsible for the X-linked form of this disease, although the precise developmental consequences resulting from the loss of this gene is not completely understood.⁵

Recently, a review revealed that hypertelorism is the most common feature in patients, being present to some degree in all individuals of both sexes. Hypospadias was also found to be present in over 82% of affected males, while cleft lip and palate was less commonly seen, reportedly only 50% of cases.⁵ A range of other craniomaxillofacial deformities has been reported in this condition and is shown in Table 1.³ Previously, there have been few reports on the overall management and long-term follow-up.^{6–9}

The aim in the present study was to review the clinical features in a cohort of patients with Opitz G BBB who are reaching skeletal maturity and evaluate the long-term results of surgical management.

METHODS

The departmental database was used to identify cases of Opitz G BBB syndrome, and case notes were retrieved, along with specific studies, including radiology and 3-dimensional computed tomographic reconstruction to assess the extent of the abnormality. Inclusion criteria selected only those cases where an experienced clinical geneticist confirmed the diagnosis.

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TABLE 1. Anomalies Associated With Opitz G BBB Syndrome³

Craniofacial	
Head	Cranial asymmetry and prominences, midline furrow, widow's peak
Facial bones	Hypoplasia
Nose	Flattened bridge, anteverted nostrils, grooved nasal tip
	Nostril cleft
Eyes	Hypertelorism, telecanthus/epicanthal folds, strabismus, entropion, ptosis
Ears	Posterior rotation
	Abnormal modeling of helix
Oral structures	Cleft lip and palate, fused teeth/supernumerary teeth
	Bifid uvula, ankyloglossia/bifid tongue
	Short lingual frenulum
Urogenital	Hypospadias, vesicoureteric reflux
Respiratory	Stridor, aspiration pneumonia, atelectasis
	Bronchiectasis, laryngotracheal cleft
	Tracheo-oesophageal cleft
Cardiovascular	ASD/VSD/PDA/TOF
	Coarctation aorta
GIT	Upper tract malformation
	Esophageal motility, imperforate anus
Others	Inguinal hernia/pes cavus/deformed digits
	Midline brain anomalies, developmental delays

ASD, atrial septal defect; PDA, patent ductus arteriosus; TOF, tetralogy of Fallot; VSD, ventricular septal defect.

Hypertelorism and cleft lip and palate (CLP) were managed according to the unit protocol^{10,11}; however, the protocol for the management of CLP was customized to deal with the management of the other associated anomalies.

RESULTS

The departmental database identified a total of 7 patients with Opitz G BBB syndrome in a 25-year period from 1980 to 2004. Of these, 5 were male and 2 female, with a current mean age of 15 years and average follow-up of 14 years. All underwent full multidisciplinary assessment with initial review by craniofacial surgeon, plastic surgeon, speech pathologist, pediatrician, pediatric surgeon, respiratory physician, and cardiologist due to the presence of multiple anomalies. Clinical genetic assessment was undertaken to establish a diagnosis, and patients were offered genetic testing. Basic radiology of skull and chest with CT scan and 3D reconstruction of the skull was performed to assess the deformity. A multidisciplinary planning was undertaken for each patient to establish treatment priority. Craniofacial anomalies were managed according to the ACFU protocol of hypertelorism and CLP.^{10,11}

On reviewing the clinical presentation, all the males were found to have hypertelorism, facial cleft, and hypospadias. Both female patients had hypertelorism and 1 female had isolated cleft of the posterior palate, but none had genitourinary anomalies. All the patients had a wide range of

clinical features. Case 7 was a mild presentation with hypertelorism, midface hypoplasia, and an associated cardiac anterior septal defect (Fig. 1A, B). Two patients were found to have MID1 gene error in X chromosome. In 4 other cases, no mutations were detected, and 1 declined testing.

The management of the anomalies of these patients was grouped in the triad of clinical features as follows.

Hypertelorism (Table 2 and Fig. 2A)

Hypertelorism was a consistent finding in all patients, and the bony interorbital distance (BIOD) was measured between the dacryons on a plain radiology of skull (Fig. 2B). Patients were grouped as mild, moderate, and severe hypertelorism on the basis of measurement. Six patients had mild and moderate, whereas 1 had severe, hypertelorism. All patients had epicanthal folds, and 2 had congenital unilateral ptosis. Three patients had congenital strabismus, and 1 acquired it following hypertelorism surgery. Two patients underwent ophthalmological correction. Four patients underwent surgery to correct the hypertelorism using box osteotomy technique preserving the cribriform plate recommended by Converse.¹² One patient required 2 procedures, a subcranial orbital osteotomy at age 5 and a box osteotomy at age 17. It was noted that there is a tendency for the cribriform plate to be low, which clearly needs to be borne in mind when planning corrective hypertelorism surgery. The pre- and post-operative BIOD were compared (Table 3). There were no significant complications following hypertelorism surgery, apart from anosmia and strabismus, which occurred on 1 occasion each.

Cleft Lip and Palate (Table 4 and Fig. 3A, B)

Out of 6 patients with cleft anomalies, 4 had bilateral complete cleft lip and palate, 1 had a unilateral complete lip and palate, and 1 patient had an isolated complete cleft of the palate. Three patients had their cleft management from birth in the ACFU, 2 patients entered the protocol at 2 and 4 years of age, respectively, and 1 patient was already skeletally mature when referred. Lip repair was performed at the average age of 6 months. This is a delay of 3 months' time of the

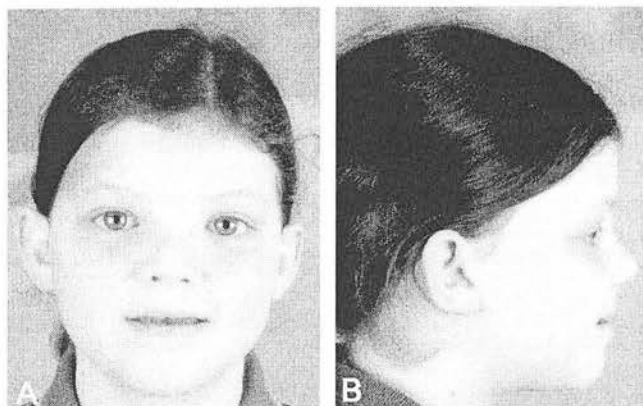


FIGURE 1. A, Eleven-year-old female with Opitz G BBB syndrome showing mild hypertelorism and nasal deformity. B, Same patient in a profile view showing midface hypoplasia.

TABLE 2. Hypertelorism

Case	Hypertelorism	Procedure	Age at Surgery
1	Mild	Box osteotomy/canthoplasty/ptosis correction	6 y
2	Moderate	Box osteotomy/canthoplasty	5 y
3	Moderate	Subcranial osteotomy and box osteotomy/canthoplasty	7 y and 17.3 y
4	Mild	Young	
5	Moderate	Box osteotomy/canthoplasty	5.2 y
6	Severe	Lost to follow-up	
7	Mild	Young	

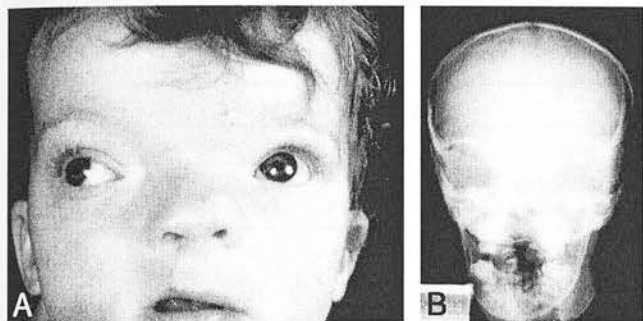


FIGURE 2. A, Severe hypertelorism and telecanthus in a 1-year-old child. B, Plain radiology of skull (AP view) for measurement of BIOD.

TABLE 3. Bony Interorbital Distance (BIOD)

Case	Preoperative BIOD	Postoperative BIOD
1	31 mm	29 mm
2	38 mm	31 mm
3	37 mm	22 mm

current CLP protocol.¹⁰ A Wardill-Kilner type of palate repair¹³ was performed in all cases, along with myringotomy and insertion of grommets at the average age of 14 months. The delay in the timing of surgery was due to the presence of respiratory, cardiac, urologic, and feeding problems that required prolonged hospitalization. Alveolar bone grafting was performed at the time of mixed dentition, similar to other patients in CLP protocol. A total of 4 out of 6 patients with CLP required superior flap pharyngoplasty at the average age of 12 years after detection of VPI on serial speech evaluation and nasoendoscopic assessment. This highlights

the importance of multidisciplinary assessment throughout the growth period. Two patients required Abbé flaps at the age of 14 and 17 years, respectively, to correct horizontal lip shortness and improve the nasolabial profile when they were adult.

Hypospadias (Table 5 and Fig. 4)

All of the males had hypospadias; 1 was perineal, 3 of penoscrotal, and 1 coronal type. Female patients did not have any genitourinary anomalies. All had a multistage repair, 2 developed fistulae requiring further surgery, and 1 had a persistent chordee. One of the patients had upper genitourinary abnormality that required ureteric surgery.

In addition to the triad of deformities, there were other associated facial anomalies. Midface deformity (Table 6), including midface hypoplasia, was present in all patients, including 1 patient who had no cleft deformity. Hypoplastic zygoma (3 out of 7 patients), depressed nasal bridge, and upturned nose (4 out of 7 patients) were also associated midface deformities.

Three patients underwent malar augmentation, 4 patients had open rhinoplasty with cantilever costochondral bone grafts, and 2 required Le Fort I maxillary advancement procedure, and these were performed after reaching skeletal maturity. One patient had good facial growth and was managed by orthodontics alone, without the need of maxillary osteotomy (Figs. 5–9). Cranial asymmetry and frontal bossing (2 patients), ear deformity, including low-set and rotated ears (3 patients), and tongue-tie (3 patients) were also identified.

Other anomalies that required appropriate management included airway problems (stridor and laryngomalacia), gastrointestinal abnormalities (imperforate anus and esophageal reflux), and cardiac diseases (PDA, ASD, VSD).

TABLE 4. Cleft Lip and Palate (CLP): Pathology and Age at Repair

Case	Pathology	Lip Repair	Palate Repair	Alveolar Bone Graft	Pharyngoplasty
1	Bilateral complete CLP	8 mo	20 mo	12 y	9 y
2	Unilateral complete CLP	5 mo	9 mo	14.5 y	7.5 y
3	Bilateral complete CLP	3 mo	10 mo	12 y	5.3 y
4	Bilateral complete CLP	7 mo	12 mo	Awaiting	—
5	Bilateral complete CLP	7 mo	10 mo	17.6 y	26.2 y
6	Isolated Cleft Palate	—	20 mo	—	—
7	No cleft	—	—	—	—

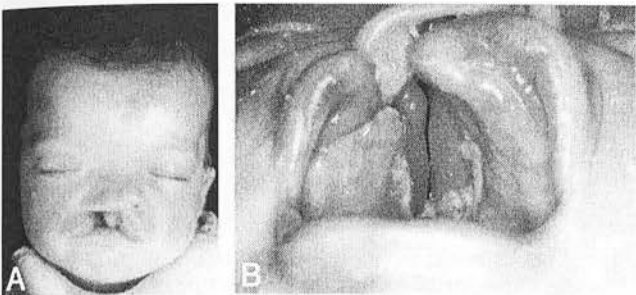


FIGURE 3. A, Five-month-old male baby showing unilateral cleft lip deformity. B, Same child showing unilateral cleft palate deformity.

TABLE 5. Hypospadias and Its Severity

Case	Hypospadias			Other Anomalies
	Perineal	Penoscrotal Penile	Coronal	
1	+			
2		+		
3			+	
4		+		VUR and UTI-Ureteric reimplantation
5		+		

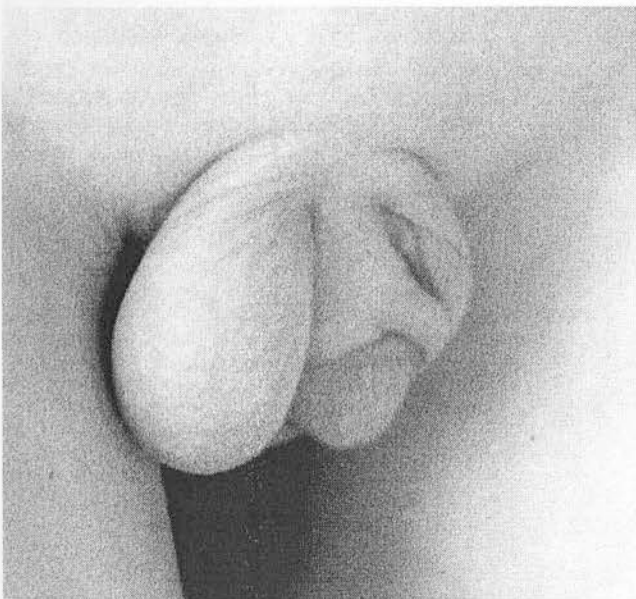


FIGURE 4. Perineal hypospadias with bifid scrotum.

All patients were regularly reviewed throughout the growth period, and the definitive correction, including orthognathic and soft-tissue refinement surgery, was performed after skeletal maturity.

DISCUSSION

Opitz G BBB syndrome is a condition with variable clinical presentation. It varies in severity from mildly affected

TABLE 6. Surgery of the Midface

Case	Malar Augmentation	Rhinoplasty	Midface Advancement
1	+	+	-
2	-	+	-
3	+	+	+
4	-	-	-
5	+	+	+
6	-	-	-
7	-	-	-

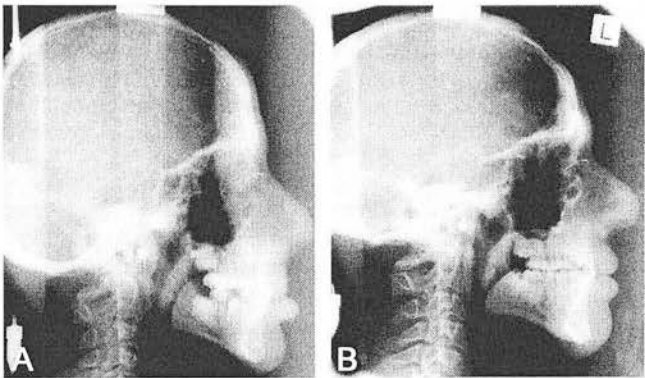


FIGURE 5. A, Lateral cephalogram in a 6-year-old male showing skeletal class III jaw relationship. B, Lateral cephalogram of the same patient after skeletal maturity at the age of 24 years.

(Fig. 1) to severely affected patients and may have multiple associated congenital anomalies. The severity may be such that neonatal death and infant mortality may occur.^{3,14} Most of the anomalies in this disorder affect the midline, suggesting that the genetic mutation exerts its effect predominantly on midline morphogenetic processes.² Investigations into the underlying genetic defects and the pathobiochemistry of this syndrome have identified that development of the ventral midline is a complicated multistep process.¹⁵

Many synonyms are attached with the Opitz G BBB syndrome, including Opitz oculogenitolaryngeal syndrome,¹⁶ Opitz syndrome,¹ and G syndrome.¹⁷ Marked overlap of the phenotype presentations, the same inheritance pattern, and male preponderance of these differently named syndromes suggested unification; hence, a compound name, Opitz G BBB syndrome, was proposed.¹⁶

The triad of deformity had variable range of presentation. Hypertelorism was present in all of the patients in this series. It has been reported that hypertelorism is a common denominator in males with identified MID1 mutations.^{4,5} BIOD measured on plain skull radiology was used to grade hypertelorism.¹⁸ All the patients in this study had hypertelorism, and these were grouped into 3 categories to highlight the variability. Tessier's classification¹² for the severity of hypertelorism could not be used because it has been described for adult population, and larger data are required for age- and gender-matched classification as described by Mulliken.¹⁹

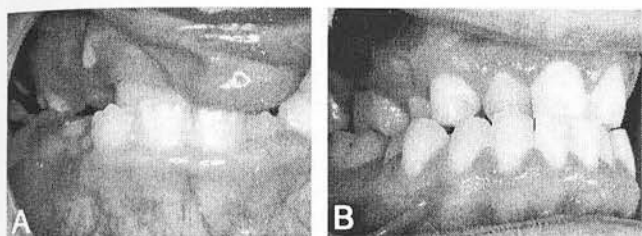


FIGURE 6. A, Occlusal relationship prior to orthodontics in the same patient in Figure 5. B, Dental relationship after completion of orthodontics management at the age of 24 years.



FIGURE 7. A, Frontal view of a 6-year-old male showing bilateral CLP, hypertelorism, and unilateral ptosis. B, Profile view of the same patient in Figure 7A.

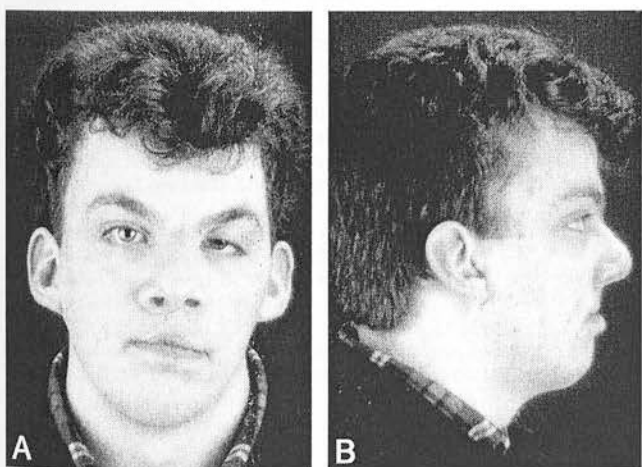


FIGURE 8. A, Same patient at age of 16 years after several reconstructive procedures. B, Profile view at 16 years of age.

Three patients had mild (30.1–34.9 mm) hypertelorism, 3 had moderate (35–39.9 mm), and 1 patient had severe (>40 mm) hypertelorism (Table 2) again showing the range of severity. This required a range of management strategy. Postoperatively, BIOD measurement was compared and showed im-

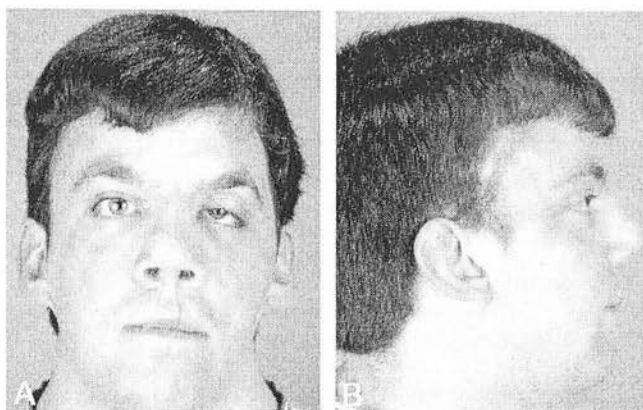


FIGURE 9. A, Same patient after skeletal maturity (24 years) showing good facial growth. B, Profile view of the same patient showing good skeletal jaw relationship after orthodontics only.

provement in all patients. All patients were evaluated by an ophthalmologist as a part of multidisciplinary assessment.

Cleft lip and palate was present in 86% in this series, and it ranged in severity from isolated cleft palate to bilateral complete cleft of lip and palate. In one report, cleft palate was seen in 34%²⁰; in another report, 34% of patients had intraoral abnormalities.⁷ In this series, the patients were managed according to the CLP protocol. However, the timing had to be altered to manage associated anomalies whose treatment was planned by multidisciplinary team. Notably, 4 out of 6 patients with cleft required pharyngoplasty, which is not commonly undertaken in our nonsyndromic isolated cleft palate population, possibly indicating the severity of midline deficiency.

Hypospadias was present in 100% of the male patients, whereas females had no genital anomalies, which is similar to the report of Peeden et al.²⁰ The types varied from distal coronal to proximal perineal with bifid scrotum, and 1 patient had associated ureteral anomaly. This contrasts the report of Peeden et al,²⁰ where the site is not recorded. Multistaged reconstruction was performed in all the patients.

There is a range of other craniofacial anomalies present in Opitz G BBB syndrome,^{1,3} including cranial asymmetry, ear malformations, midface hypoplasia, and nasal deformity. In this study, midface hypoplasia was present in all patients, including 1 without any cleft. The common procedures performed in these patients are augmentation rhinoplasty, malar augmentation, and Le Fort I maxillary advancement. One patient in this series with bilateral CLP had good facial growth throughout the developmental period and achieved a good facial balance with orthodontics alone, as shown in Figures 5 and 6.

The associated anomalies previously described include neuromuscular defects of the esophagus, imperforate anus, mental retardation and dysphagia with aspiration, laryngotracheoesophageal anomalies, central nervous system malformations, and cardiac anomalies.^{2,21}

In this series, 51% had gastrointestinal anomalies, whereas 43% had cardiac anomalies. Four patients (57%) had

neonatal respiratory illnesses causing long periods of hospitalization, while only 1 patient had laryngomalacia. Presence of these multiple diseases was the reason for delay in cleft repair. These visceral abnormalities are the cause of high morbidity and mortality in patients with Opitz G BBB syndrome.^{2,14}

In conclusion, the condition has a triad of clinical features with variable severity, which have been highlighted in this series. Within the triad, the features have a range of presentation that may be associated with other anomalies. This results in our recommendation that management by multidisciplinary team is required for customized treatment and coordinated care.

Finally, we propose a management strategy (Table 7) recommending that patients with Opitz G BBB syndrome are best treated in regional craniomaxillofacial centers so that an appropriate customized management protocol can be followed for each individual patient.

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TABLE 7. Protocol for the Management of Opitz G BBB Syndrome

Period of Growth	Management
Neonatal period	Multidisciplinary assessment by craniofacial surgeon, ENT, ophthalmologist, pediatrician, pediatric urologist, cardiologist, respiratory physician, speech pathologist, and clinical geneticist
Childhood period	Hypertelorism: ophthalmology assessment Neurosurgery assessment Surgery timing influenced by the developing dentition CLP: lip repair and myringotomy, palate repair Speech review Dental and orthodontic evaluation Hypospadias: urological assessment 3-Stage reconstruction Management of cardiac, respiratory and gastrointestinal anomaly if indicated
Adulthood	Orthognathic and soft tissue refinement surgery

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From Birth to Maturity: A Group of Patients Who Have Completed Their Protocol Management. Part II. Isolated Cleft Palate

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Background: The optimal management of the cleft palate patient from birth to completion of treatment continues to present a formidable challenge to the plastic surgeon. The management by multidisciplinary teams is well established, but long-term outcome data of cases managed by protocol remain sparse. This study continues to present the results of the Australian Craniofacial Unit of patients with isolated cleft palate who completed protocol management at the unit under the care of the senior author (D.J.D.) during the 29-year period from 1974 to 2003.

Methods: A retrospective study of the outcomes in relation to facial growth, speech, hearing, and occlusion is presented of patients with an isolated cleft palate.

Results: Thirty-two cases were identified from the departmental database, involving 17 female patients and 15 male patients. Cephalometric analysis at skeletal maturity revealed a range of facial growth, and maxillary advancement surgery was deemed necessary in just two cases. Speech results were evaluated using speech therapy assessments during development and at maturity. At maturity, 18 of 32 patients were assessed as being within normal limits. The hearing was within -20 dB, with just two exceptions.

Conclusion: Overall, these is a range of outcomes, but the results confirm that facial growth does not appear to be adversely affected by use of the pushback technique to reconstruct the palate. (*Plast. Reconstr. Surg.* 117: 515, 2006.)

The optimal management of patients with a cleft palate remains debatable. However, the ideal aims of normal speech and hearing, normal occlusion, and with a normal facial appearance and psychological well-being are unlikely to be achieved without multidisciplinary management.¹

However, as we have previously highlighted, there has been little published regarding the outcome of multidisciplinary treatment of cleft lip-cleft palate patients.² This report attempts to address some of the uncertainties in the management of isolated cleft palate, particularly the effect of palatal surgery on subsequent facial growth.

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PATIENTS AND METHODS

The Australian Craniofacial Unit in Adelaide has been managing cleft lip-cleft palate patients with a consistent multidisciplinary treatment protocol since 1974² under the direction of the senior author (D.J.D.). This is summarized in Table 1. The patients who met the entry criteria of completing protocol management had their case notes and radiographs examined and the results recorded.

Birth

All patients and parents are evaluated by the plastic surgeon and the speech pathologist as soon as possible after birth. The parents are educated about details of the proposed management of the cleft palate by a dedicated multidisciplinary team, with the emphasis on the long-term nature of this program. Feeding advice is provided by the team speech pathologist.

Preoperatively, the patient is assessed again by the speech pathologist, along with the otolaryn-

Table 1. Australian Craniofacial Unit Cleft Lip–Cleft Palate Consultation Protocol

Newborn	
	An initial appointment with the surgeon and the speech pathologist will be made shortly after birth; the speech pathologist will continue to monitor the feeding process
Three months	
	Surgeon: Consultation regarding management process
	Speech pathologist: Review feeding and discuss speech management
	Otolaryngology: Otoscopic examination
	Pedodontist: Discuss dental development and management
	Clinical genetics: Assessment for other anomalies, future pregnancies
	Photography: Photographic records
	Craniofacial registrar: To record all relevant medical data on protocol form
First year	
	Surgeon: Review at 1 yr
	Speech pathology: Review at 1 yr to assess speech and language development
	Orthodontics: Review and discussion regarding future management
	Audiology: Hearing assessment
	Otolaryngology: Assessment 6 mo after myringotomy insertion or if not required, review 1 yr
	Photography: At 1 yr
Second to fifth years	
	Surgeon: Annual review; nasendoscopy at age 5 yr if indicated
	Speech pathology: Assessment at 1.5, 2.5, 3.5, and 4.5 yr
	Orthodontics: Review and discussion regarding future management
	Audiology: Review as indicated
	Otolaryngology: Annual review
	Pedodontics: Annual review
	Multidisciplinary cleft lip–cleft palate clinic: 1.5 and 4.5 yr
Sixth to 12th years	
	Surgeon: Annual review; nasendoscopy if indicated
	Speech pathology: Assessment at 8 and 12 yr
	Orthodontics: Annual review
	Audiology: Review as indicated
	Otolaryngology: Annual review
	Radiographs: Cephalometric studies annually
	Multidisciplinary cleft lip–cleft palate clinic: 8 and 12 yr
Thirteenth year to skeletal maturity	
	Surgeon: Annual review; nasendoscopy if indicated
	Speech pathology: Review as indicated
	Orthodontics: Assess growth, plan any orthognathic surgery
	Pedodontics: Annual review
	Final assessment: Cephalogram and photographs before discharge

gologist, the pedodontist, and the surgeon. Pre-operative photographs are obtained.

First Year

The cleft palate repair was undertaken between 6 months and 38 months using a modified two-flap pushback palatoplasty (Veau-Wardill-Kilner type). The procedure is modified in that the

two flaps are extended to include the anterior palate as described by Bardach.³ At the same anesthetic, otologic examination is undertaken and myringotomy tubes inserted as necessary. Oral fluids can be recommenced immediately after surgery. Arm splints are worn on an as-needed basis. Patients are examined by the plastic surgeon 1 week after surgery and again at 6 weeks. At this time, review by the otolaryngologist is undertaken if myringotomy tubes have been inserted. All cases are reviewed at 6-month intervals until the ears are normal and myringotomy tubes are no longer required.

A review by the speech pathologist is undertaken to evaluate speech and language development. Review by orthodontics is also undertaken to outline the protocol treatment program during childhood.

Second through Fifth Years

Annual review by the plastic surgeon is undertaken. Pedodontic and audiology examinations are undertaken annually. Speech assessment is undertaken at 1.5, 2.5, 3.5, and 4.5 years of age. If necessary, nasendoscopy can be performed after the fifth year. The cleft lip–cleft palate multidisciplinary team meeting is attended at 1.5 and 4.5 years.

Sixth through Twelfth Years

Annual review by the plastic surgeon is performed. Otolaryngologic review is performed every 6 months if myringotomy tubes were placed; otherwise, every 12 months. Speech is assessed at 8 and 12 years of age and nasendoscopy performed if necessary. Orthodontic review is performed annually to monitor facial growth, with the occlusion including review of cephalometric radiographs. The multidisciplinary team meeting is attended at ages 8 and 12 years.

Audiometry

Audiometric findings are assessed according to the following criteria: hearing levels of less than 20 dB are considered to be in the normal range, mild loss is between 20 dB and 30 dB, and significant loss is considered to be greater than 30 dB.

Orthodontic Treatment

Assessment at age 4.5 years is performed when the primary dentition is complete. At age 6 years, study models are used to record and assess the

Table 2. The Isolated Cleft Population

Case	Sex	Cleft Type	Age at Repair (mo)
1	F	S	9
2	M	S	9
3	M	S	9
4	F	S	7
5	F	S	11
6	M	S	12
7	F	S	11
8	M	H + S	12
9	M	H + S	13
10	F	H + S	9
11	M	H + S	18
12	F	H + S	10
13	F	H + S	13
14	M	H + S	11
15	F	H + S	14
16	M	PR	14
17	F	PR	14
18	M	PR	13
19	F	PR	13
20	F	PR	18
21	F	PR	14
22	F	PR	12
23	M	PR	8
24	M	PR	12
25	M	SMCP	38
26	M	SMCP	32
27	F	SMCP	8
28	F	SMCP	26
29	M	SMCP	37
30	F	SMCP	25
31	F	SMCP, VDW	36
32	M	SMCP, chromo	11

F, female; M, male; S, cleft soft palate only; H + S, cleft hard and soft palate; PR, Pierre Robin sequence; SMCP, submucous cleft palate; VDW, Van der Woude; chromo, chromosomal anomaly.

developing occlusion, and radiographs assess the number, morphology, and position of the developing secondary dentition.

Table 3. Cephalometric Analysis at Skeletal Maturity for Nonsyndromic Isolated Cleft Palate Patients

Case	Sex	Age (yr)	SNA	SNB	ANB
1	F	18	77	72	5
2	M	17	84	82	2
3	M	17	82	80	2
4	F	17	78	76	2
5	F	16	79	79	0
6	M	18	83	77	6
7	F	16	79	72	7
8	M	18	71	67	4
9	M	16	81	80	1
10	F	17	75	73	2
11	M	20	70	68	2
12	F	17	78	78	0
13	F	17	74	75	-1
14	M	19	79	77	2
15	F	17	75	81	-6

F, female; M, male; SNA, angle on the cephalogram made by the sella, the nasion, and point A on the maxilla; SNB, angle on the cephalogram made by the sella, the nasion, and point B on the mandible; ANB, angle on the cephalogram made by point A, the nasion, and point B.

Table 4. Cephalometric Analysis at Skeletal Maturity for Pierre Robin Sequence Patients

Case	Sex	Age (yr)	SNA	SNB	ANB
16	M	15	70	68	2
17	F	18	79	76	3
18	M	18	84	78	6
19	F	16	79	80	-1
20	F	18	78	77	1
21	F	18	81	77	4
22	F	17	74	73	1
23	M	16	85	78	7
24	M	16	73	73	0

F, female; M, male; SNA, angle on the cephalogram made by the sella, the nasion, and point A on the maxilla; SNB, angle on the cephalogram made by the sella, the nasion, and point B on the mandible; ANB, angle on the cephalogram made by point A, the nasion, and point B.

At approximately 13 years of age, full orthodontic treatment is undertaken to align the dental arches. Assessment of future facial growth and the need for orthognathic correction is undertaken. This early assessment is essential if appropriate orthodontic therapy is to begin.

Orthognathic Surgery

At approximately 17 or 18 years, final assessment of the facial pattern is carried out. Cephalometric study aids the planning of orthognathic surgery, and hand/wrist radiographs confirm that growth is complete. If maxillary advancement is required, overcorrection allows for inevitable relapse. Orthodontic treatment is continued to confirm that the occlusion is functional and aesthetic once any final fine adjustments following surgery have been completed.

Speech Evaluation

Speech analysis is based on patient review and recorded speech samples. All the evaluations have been performed under the supervision of two se-

Table 5. Cephalometric Analysis at Skeletal Maturity for Submucous Cleft Palate Patients

Case	Sex	Age (yr)	SNA	SNB	ANB
25	M	17	82	80	2
26	M	17	88	88	0
27	F	15	77	73	4
28	F	16	82	83	-1
29	M	16	85	87	-2
30	F	15	90	85	5
31	F	18	87	82	5
32	M	17	73	75	-2

F, female; M, male; SNA, angle on the cephalogram made by the sella, the nasion, and point A on the maxilla; SNB, angle on the cephalogram made by the sella, the nasion, and point B on the mandible; ANB, angle on the cephalogram made by point A, the nasion, and point B.

Table 6. Outcomes: Otolaryngology, Speech, and Orthodontics

Case	Grommets	Hearing	Pharyngoplasty	Speech Outcome/Therapy	Occlusion/ Orthognathic Surgery	Total Operations
1	N	N	N	N; therapy for resonance	I/N	1
2	N	N	N	N; therapy for articulation	I/N	2
3	1	N	N	N	I/N	2
4	2	N	N	N	I/N	3
5	4	N	N	N	I/N	5
6	3*	N	N	N	I/N	4
7	1	N	N	N; therapy for resonance	I/N	2
8	1	-30 dB, right	N	Mild lateralization S/Z; therapy for articulation	I/N	3
9	1	N	N	Mild lateralization S/Z	II/N	3, fistula × 1
10	5*	-40 dB, left	N	N	I/N	7, tympanoplasty
11	3	N	O, age 10; revision, age 12	Mild VPI; therapy for articulation	I/N	6
12	2	N	N	Mild VPI; therapy for articulation	I/N	4, tympanoplasty
13	3*	N	N	Mild VPI	I/N	5, tympanoplasty
14	2	N	Sup. flap, age 6; I, age 11	VPI; therapy declined	I/N	5
15	4	N	Sup. flap, age 14	VPI; therapy for articulation	I/Y	7, LF 1 adv 8 mm, Imp 2 mm
16	3	N	N	VPI; therapy declined	II/Y	6, temporomandibular joint reconstruction at age 5; BSSO adv 6 mm
17	1	N	N	Mild VPI; therapy for resonance	I/N	2
18	N	N	O, age 10; I, age 8; I, age 11; O, age 17	N; therapy for resonance	I/N	5
19	3	N	N	Mild lateralization S/Z; therapy for articulation	I/N	4
20	N	N	N	N	I/N	1
21	1	N	I, age 10	N; therapy for VPI	I/N	3
22	N	N	I, age 11	N; therapy for VPI	I/N	4, fistula × 2
23	2	N	O, age 10; revised, age 17	Mild VPI, therapy for VPI	I/N	5
24	N	N	N	N; therapy for VPI	II/N	1
25	N	N	O, age 4; revised, age 16	De-nasal speech; therapy for resonance	I/N	3
26	N	N	N	N; therapy for language	III/N	1, language disability
27	N	N	N	N	I/N	1
28	3	N	N	Mild VPI, therapy declined	I/Y	5, BSSO 6 mm adv
29	3	N	I, age 8	N; therapy for VPI	I/N	4
30	1	N	N	N; therapy for language	I/N	2
31	2	N	N	N; therapy for resonance	III/N	3
32	3*	N	Sup. flap age 14	Moderate VPI; therapy for language	I/Y	6, LF 1 adv 10 mm, genioplasty

O, Otorrhea; Sup. flap, superior pharyngeal flap; I, posterior pharyngeal wall implant; velopharyngeal insufficiency; Imp, impaction; BSSO, bilateral sagittal split osteotomy; LF, Le Fort; adv, advancement; N, not required; S/Z, verbal sounds.

*Long-term myringotomy tubes fitted.

nior speech pathologists with extensive experience of managing cleft lip–cleft palate patients.

Speech was evaluated on articulation, velopharyngeal competence, and nasality (resonance) and intelligibility. The outcome at skeletal maturity is given a speech rating as follows: within normal limits, mild, or severe, where the latter two are assigned details of the abnormality.

Articulation is recorded as having structural deficit or compensatory changes. Nasal resonance is rated as normal, hyponasal, or hypernasal. Nasendoscopy supplemented with videofluoroscopy is performed if velopharyngeal dysfunction is suspected. This can be performed after 5 years of age. Dynamic testing is carried out using simultaneous lateral videofluoroscopy using a 30-degree nasendoscope, as we have previously described.⁴ For those cases that require interventional therapy, the component of speech that is anomalous is recorded.

RESULTS

Thirty-two patients with isolated cleft palate were found to fall within the strict selection criteria for entry into the study. This included 17 female patients and 15 male patients. The entry criteria included that the senior author (D.J.D.) treated these cases from birth and that the subsequent management was supervised by the cleft lip–cleft palate team using the Unit's multidisciplinary protocol. The current age range of the study group is from 16 to 29 years.

The 32 cases can be subdivided into a number of different groups depending on the severity of their cleft. There are 15 nonsyndromic isolated cleft palates (seven of whom had clefts affecting the soft palate only and eight of whom had clefts affecting both the hard and soft palates). An additional eight cases were diagnosed by clinical genetics as Pierre Robin sequence. In addition, six were nonsyndromic submucous cleft palates, and an additional two cases of submucous cleft palate had the anomaly as part of a syndrome, one each of Van der Woude syndrome and trisomy 18 syndrome.

The cleft palates were all repaired between 7 months and 38 months. The protocol for the timing of the repair was changed from 9 months to 6 months in 1984, in line with the practice of other surgeons at this time who wished to optimize speech outcome. The causes for delay beyond the protocol timing for repair included illness and scheduling requests. In particular subgroups, the timing was later both in the submucous cleft palates where there was often a delay in diagnosis and

in the Pierre Robin cases where the palatal closure was delayed as part of airway management. No cases required reoperation for hemorrhage. All cases required orthodontic management.

Hearing

Hearing was within the defined normal limits for all but two patients. One patient was severely impaired on one side (−40 dB) and required a hearing aid, and the other patient also had a unilateral loss; in this case, −30 dB.

However, 23 of 32 cases required myringotomy insertion for middle ear effusion, and all the cleft subgroups were affected (Table 2). Interestingly, 16 of the 32 cases required multiple operations. These were all undertaken before the age of 10 years except one (case 15); this patient developed middle ear effusion following maxillary advancement osteotomy and required myringotomy tubes on two occasions following her maxillary surgery. Curiously, this patient had no ear problems in the 8 years preceding her orthognathic surgery. Four patients required long-term ventilation tubes and two patients required reconstructive tympanoplasty.

Table 7. Speech Anomalies during Growth

Case	Articulation	VPI	Resonance
1	No	Yes	Yes
2	Yes	No	Yes
3	No	Yes	Yes
4	No	Yes	Yes
5	No	Yes	Yes
6	No	Yes	Yes
7	No	Yes	Yes
8	Yes	Yes	Yes
9	Yes	No	Yes
10	No	No	No
11	Yes	Yes	Yes
12	Yes	Yes	No
13	No	Yes	Yes
14	Yes	Yes	Yes
15	Yes	Yes	Yes
16	No	Yes	No
17	No	Yes	Yes
18	No	Yes	Yes
19	Yes	Yes	Yes
20	No	Yes	Yes
21	No	No	Yes
22	No	Yes	Yes
23	No	Yes	Yes
24	No	Yes	Yes
25	No	Yes	Yes
26	Yes	Yes	Yes
27	No	No	No
28	No	Yes	Yes
29	No	Yes	Yes
30	Yes	Yes	Yes
31	No	Yes	Yes
32	Yes	Yes	Yes

VPI, velopharyngeal insufficiency.

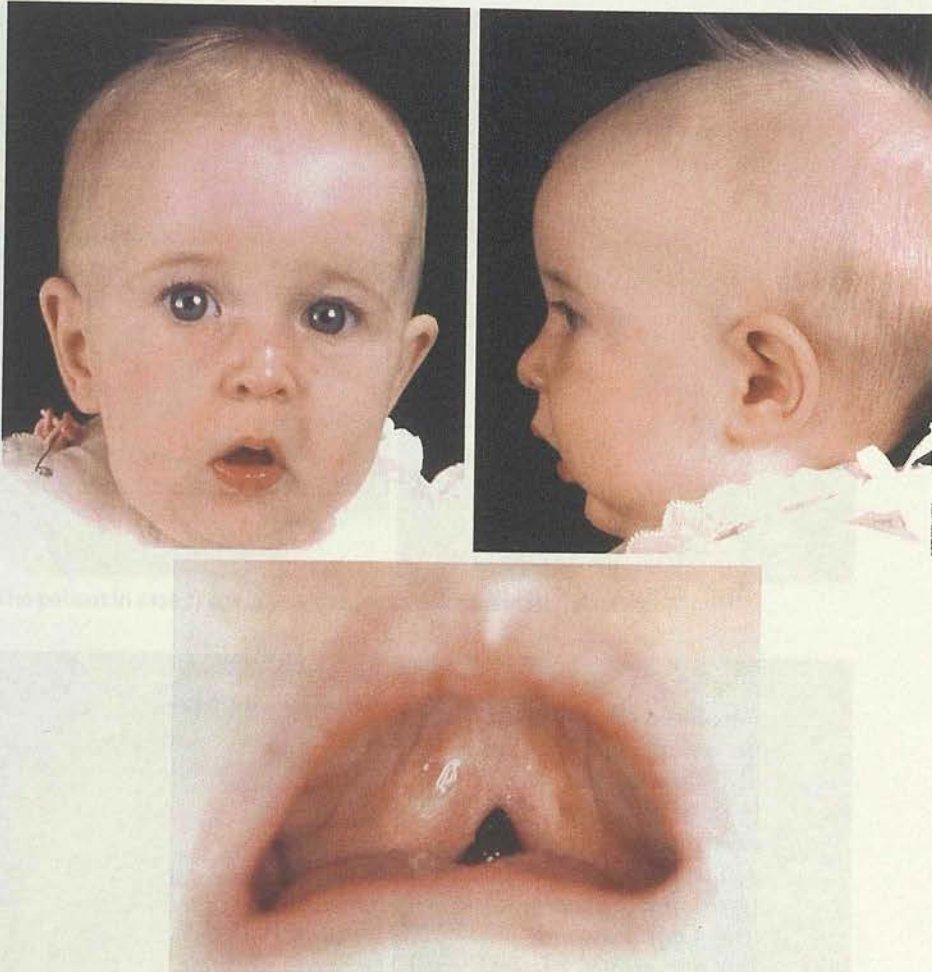


Fig. 1. (Above, left and right) The patient in case 7; preoperative age, 3 months; anteroposterior and lateral views. (Below) The unoperated cleft affecting the soft palate.

Orthognathic

Four of 32 patients underwent orthognathic surgery. All four subgroups were affected. Of these, one underwent maxillary advancement alone, whereas one case required bimaxillary (advancement) orthognathic surgery. In addition, two patients required mandibular surgery alone. One case underwent genioplasty 3 months after bimaxillary surgery. The one case requiring advancement also required augmentation of the maxillary eminences with onlay bone grafts. Of all the patients with nonsyndromic isolated cleft palate, just a single case required maxillary advancement. The cephalometric analyses at skeletal maturity before any orthognathic surgery was undertaken are shown in Tables 3 through 5.

Speech

The speech outcomes were that 18 of the 32 cases were judged to be within normal limits at skeletal

maturity (Table 6). Some of these cases judged to be within normal limits still had persistent but minor errors. Although 11 of the 17 female patients (65 percent) achieved normal speech, only seven of the 14 male patients (50 percent) managed to attain this. However, using Fisher's exact test, this was not statistically significant. Overall, the speech outcome did not appear to be related to any of the cleft palate subgroups in this series.

Speech therapy was recommended for 23 of the 32 patients (72 percent) at some time during their management (Table 6). This applied to 11 of 17 female patients and 12 of 15 male patients. Again, using Fisher's exact test, there was no statistically significant differences between the sexes. Three cases declined therapy, and the distance from home to the treatment center was a factor in all of these cases.

Within the female group, 10 cases underwent therapy. These included a range of speech anom-



Fig. 2. The patient in case 7; age, 5 years; anteroposterior and lateral views.



Fig. 3. The patient in case 7; age, 10 years; anteroposterior and lateral views.

alies. The indications for therapy were resonance in four cases, articulation in three cases, velopharyngeal insufficiency in two cases, and language one case. Within the male cohort, there was a range of indications for intervention, and in total, 10 cases underwent therapy. The indications were articulation in three cases, velopharyngeal insufficiency in three cases, resonance in two cases, and language in two cases. The speech pathology will now be considered in more detail, reviewing in turn articulation, velopharyngeal insufficiency, and resonance.

Starting with articulation, it is readily apparent that during the period of management, the presence of articulation errors was fairly common, with 11 of the 32 patients affected (Table 7). There are no differences between the sexes. Most of these (five of 11 cases) had associated problems of neuromotor dysfunction or hearing loss. The term "neuromotor dysfunction" was applied to cases of slow motor development, with resulting hypotonia, which affected velopharyngeal integrity. Of the remainder, three cases had compensatory patterns associated with velopharyngeal insufficiency,



Fig. 4. (Above, left and right) The patient in case 7; age at skeletal maturity, 16 years, at completion of the protocol management. (Below, left) The dental occlusion at completion of orthodontics. (Below, right) The appearances of the repaired palate.

and an additional three cases had S/Z lateralization.

Velopharyngeal insufficiency was very common during childhood. It occurred in 27 of 32 cases (86 percent) at some point during development and occurred equally in both the male and female group. However, there was a tendency for velopharyngeal insufficiency to present in boys at approximately 3 years of age, whereas it appeared later in girls, at approximately 12 years of age. Management included therapy, pharyngoplasty, or tonsillectomy.

Ten of the 32 patients underwent pharyngoplasty or augmentation of the posterior pharyngeal wall with an implant. Four of the 10 patients had a single intervention, whereas the remaining six patients required multiple interventions, the maximum being four interventions in the patient

in case 18. In total, 16 operative interventions were recorded. The first intervention was an orticochea pharyngoplasty in four cases, a superiorly based pharyngeal flap in three cases, and a pharyngeal implant to augment the posterior pharyngeal wall also in three cases. The distribution of the cases requiring pharyngoplasty was as follows: three cases had isolated clefts of both the hard and soft palates, three were in the Pierre Robin group, one was submucous cleft palate, and the remaining case (case 32) had a syndromal chromosomal anomaly with intellectual delay.

The outcome after pharyngoplasty was that three cases achieved speech judged to be within "normal limits." Of the remaining cases, two were almost normal. The remaining four cases had different anomalies: hypernasality and air escape; lateralization of sibilants; and finally the worst case

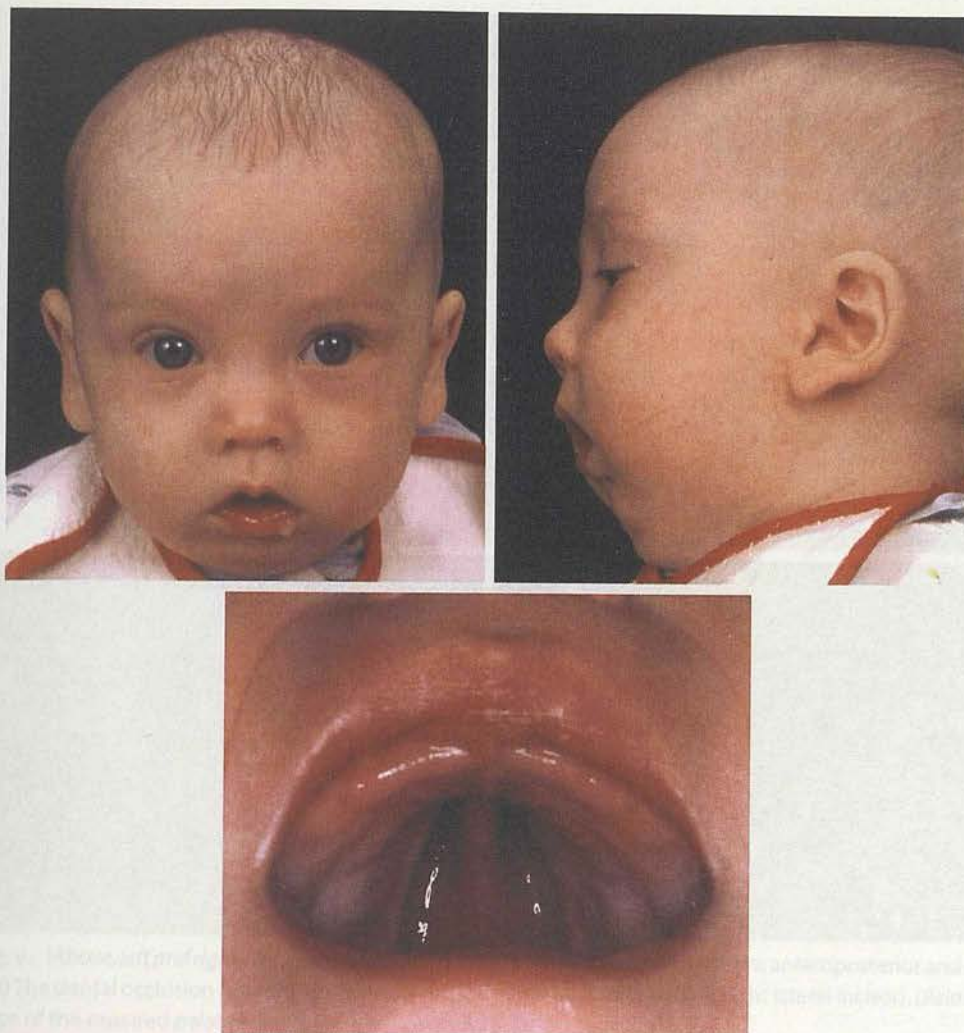


Fig. 5. (Above, left and right) The patient in case 19; preoperative age, 6 months; anteroposterior and lateral views. (Below) The unoperated wide cleft palate associated with Pierre Robin sequence.

(case 32), a patient who had difficulty with articulation and was only moderately intelligible with persistent nasal escape. Regarding the two cases with less than normal speech who declined pharyngoplasty, in one (case 13) the speech was judged to have mild audible escape, and the other (case 16) had moderate velopharyngeal insufficiency. Overall, judging the outcome of all 32 cases, even those in which the speech results were judged to be within normal limits, many still had mild articulation errors.

Finally, again reviewing all 32 cases, resonance was similarly very common at some stage during development in both male and female patients, with 28 of the 32 patients affected. In female patients, two cases were unaffected, 11 of 17 (64 percent) were judged to have a mild anomaly, and four of 17 (24 percent) were moderately or se-

verely affected. In male patients, two were unaffected, seven of 15 were mildly affected, and four of 15 (27 percent) were moderately or severely affected.

DISCUSSION

The management of cleft palate from birth to skeletal maturity requires an enormous amount of resources and dedication by a multidisciplinary team. We believe that optimal management is achieved by the adherence to treatment protocols by a dedicated multidisciplinary team.

The patients in this cohort have all been managed by a single surgeon (D.J.D.) and reflect a group treated by protocol management. This cohort is continually increasing as further cases attain maturity.

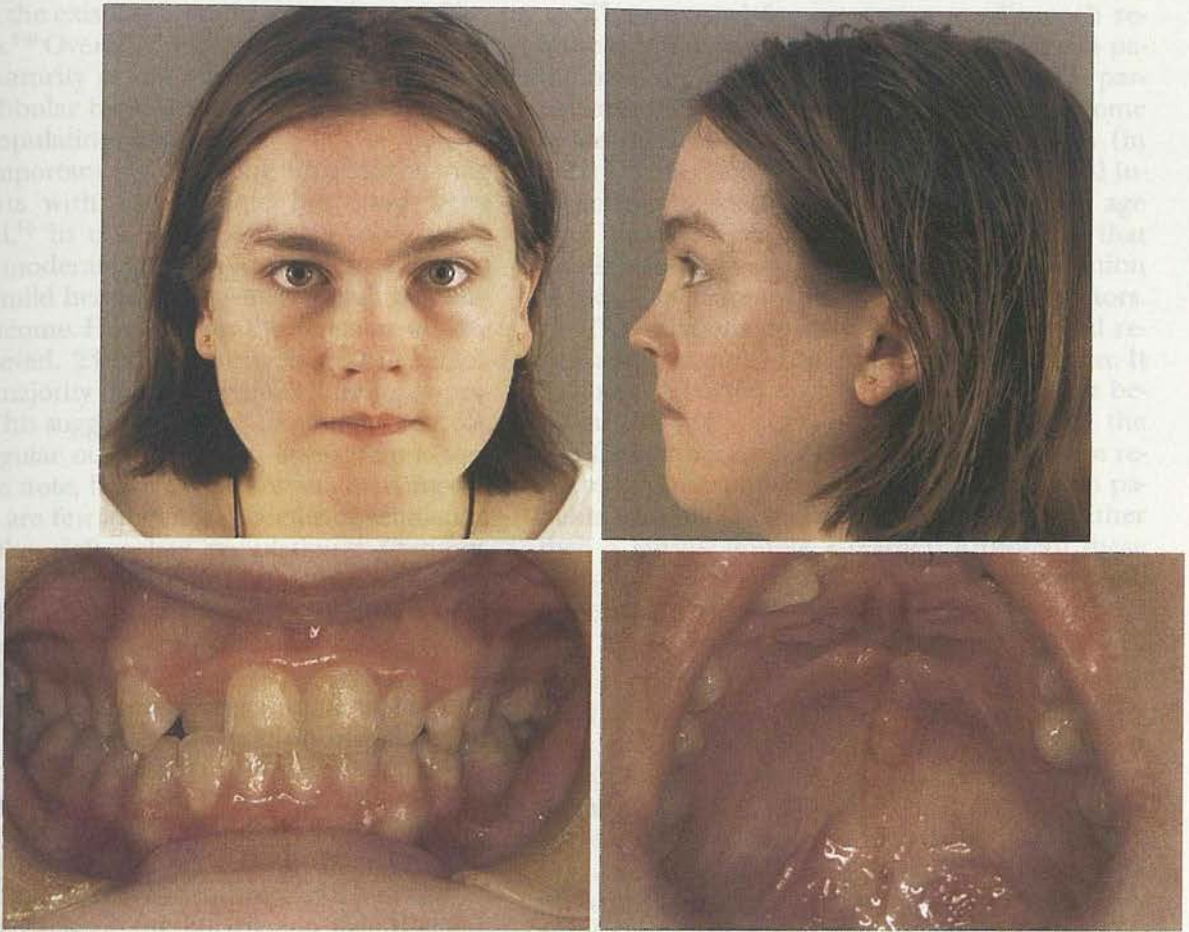


Fig. 6. (Above, left and right) The patient in case 19 at skeletal maturity; age, 16 years; anteroposterior and lateral views. (Below, left) The dental occlusion (she did not want correction of the instanding upper right lateral incisor). (Below, right) The appearance of the repaired palate.

Reviewing these outcomes, it is apparent that the amount of facial growth is unpredictable and does not correlate with the anatomical severity of the cleft. The low incidence of orthognathic surgery (in a unit where orthognathic surgery is routinely undertaken in appropriate clinical situations), combined with the results of the cephalometric studies, suggests that there is not an association between the method of repair (two-flap pushback technique) and the inevitable development of maxillary hypoplasia. It has been suggested that the two-flap pushback method of palate repair be abandoned because of the effect on subsequent midface growth.⁵ This report also confirms another recent study suggesting that satisfactory long-term mid facial growth can be achieved using this method of cleft palate repair⁶ (Figs. 1 through 6). The reliability of this method of palate repair is apparent from the low fistula rate, with just two of 32 patients requiring an additional operative intervention for this.

It is also interesting to compare the low incidence of orthognathic surgery in this study with that of the previously published study of complete unilateral cleft lip–cleft palate,² which similarly found a low incidence of orthognathic surgery at skeletal maturity. The final midface growth at skeletal maturity appears to be difficult to predict. Examination of the different subgroups in this series fails to identify any relationship between midface growth and the severity of the original cleft. Review of the Pierre Robin subgroup is particularly interesting because only one case (case 16) required orthognathic surgery. This patient had undergone temporomandibular joint reconstruction in childhood following infection, which clearly would have influenced subsequent facial growth. Significantly, detailed cephalometric analysis of the mandible at skeletal maturity found no evidence that the Pierre Robin group ultimately had significantly smaller mandibles when compared with nonsyndromic cleft palate cases,

given that the existing literature contains conflicting results.⁷⁻⁹ Overall, the cephalometric studies at skeletal maturity reveal a tendency for maxillary and mandibular hypoplasia when compared with normal population values.

The importance of achieving adequate hearing in patients with cleft palate has long been recognized.¹⁰ In our series, only one case had a unilateral moderate hearing loss and another had unilateral mild hearing loss, which overall is a satisfactory outcome. However, for this outcome to have been achieved, 23 of 32 cases required myringotomy, the majority of whom required multiple procedures. This suggests that our treatment protocol, with its regular otolaryngologic assessment is warranted. We note, however, that others have argued that there are few long-term benefits of ventilation tubes in the cleft palate population.¹¹ Our outcomes also confirm a recent long-term study that concluded that conservative management of otitis media in cleft palate was associated with a poorer outcome.¹²

Speech outcome was judged to be within normal limits in 18 of 32 cases overall. However, all the different cleft palate subgroups had patients who had suboptimal speech. The worst two cases of speech outcome (cases 15 and 32) both had poor neuromuscular function.

Pharyngoplasty was required by 10 of the 32 cases, and each of the subgroups was affected. However, most of these cases occurred in the Pierre Robin group. However, examination of the data revealed that most cases requiring a pharyngoplasty were treated in the early part of this study and that this has become less frequently required. It is notable that there were other adverse factors present in those patients requiring multiple surgical interventions for pharyngoplasty, which included hearing impairment, late repair, and intellectual delay.

The use of nasendoscopy has allowed dynamic visualization of the velum and pharyngeal wall and resulted in the tailoring of pharyngeal surgery. This policy has resulted in the use of orticochea, superior pharyngeal flap pharyngoplasties, and posterior pharyngeal wall implants when indicated. Examples of each of these types of pharyngoplasty required subsequent modification on occasion. The posterior wall implant only required revision in one case, and we speculate that is attributable to our use of preoperative marking during nasendoscopy.¹³ The implants used were of homologous costal cartilage, which have been reported by others to be a good option,¹⁴ and were not associated with any operative complications.

The protocol for management of speech requires regular review by the dedicated speech pathologist during childhood. We think this is particularly important, as speech does not become stable until 10 years of age.¹⁵ This accounts (in part) for the fact that cases of velopharyngeal insufficiency treated conservatively up to this age may improve spontaneously. We speculate that stabilization of hearing function and maturation of motor speech may also be contributing factors.

The relationship between type of surgical repair and outcome of speech remains uncertain. It has been reported that there is no difference between the two-flap pushback approach and the von Langenbeck repair,¹⁶ whereas others have reported significantly better speech outcome in patients who had a two-flap pushback repair rather than a Furlow double Z-plasty.¹⁷ Although these cases have produced an overall satisfactory result, some disappointing outcomes remain.

CONCLUSIONS

The outcome of treatment at skeletal maturity by a protocol management and dedicated cleft team, with surgery undertaken by a single surgeon, of a cohort of isolated cleft palate cases is presented. The results presented are encouraging, and further cases will provide additional data while review and refinement of the treatment protocol continues.

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From Birth to Maturity: A Group of Patients Who Have Completed Their Protocol Management—Part III. Bilateral Cleft Lip–Cleft Palate

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Background: The optimal management of cleft lip–cleft palate patients presents a formidable challenge to the cleft surgeon. This is especially so in the case of bilateral cleft lip–cleft palate, and the long-term management in a multidisciplinary setting is essential. This study presents the results of the specific management protocol at the Australian Craniofacial Unit for patients with bilateral cleft lip–cleft palate who have completed their protocol treatment under the care of a single surgeon (D.J.D.) during the period 1974 to 2006.

Methods: A retrospective study of the outcomes in relation to facial growth, speech, hearing, and occlusion is presented of patients with bilateral cleft lip–cleft palate.

Results: Nineteen cases were identified from the departmental database, 12 male patients and seven female patients. Six patients with severe craniofacial deformities who had bilateral cleft lip–cleft palate were excluded. Cephalometric analysis at skeletal maturity identified that a majority of cases had midface hypoplasia requiring midface advancement in 14 cases. Speech and hearing outcomes were worse when compared with other clefting types.

Conclusion: Overall, these results demonstrate that facial growth is more affected in bilateral cleft lip–cleft palate patients than in either unilateral cleft lip–cleft palate or isolated cleft palate patients. (*Plast. Reconstr. Surg.* 128: 1, 2011.)

CLINICAL QUESTION/LEVEL OF EVIDENCE: Therapeutic, IV.

The Australian Craniofacial Unit in Adelaide has been managing cleft lip–cleft palate patients with a consistent multidisciplinary treatment protocol since 1974 under the direction of the senior author (D.J.D.). The protocol is summarized below.

From 1974 to 2006, there were a total of 19 patients who had completed their management protocol from birth to adulthood. The records of these 19 patients, including case notes, photographs, and radiographs, were examined and the notes recorded. Two representative cases are illustrated in Figures 1 through 8.

PATIENTS AND METHODS

Birth

As soon as possible after birth, the patient and parents are evaluated by the cleft surgeon and the speech pathologist. The parents are educated about the details of the proposed protocol management of the bilateral cleft lip–cleft palate, with an emphasis on the long-term management from birth to adulthood. Feeding advice is given by the Unit's speech pathologist and, importantly, the speech pathologist follows up the patient on discharge from the maternity ward and is able to offer help and advice either in person or by telephone.

Preoperatively, the patient is again assessed by the speech pathologist along with the orthodontist and the cleft surgeon. Preoperative photographs are also obtained.

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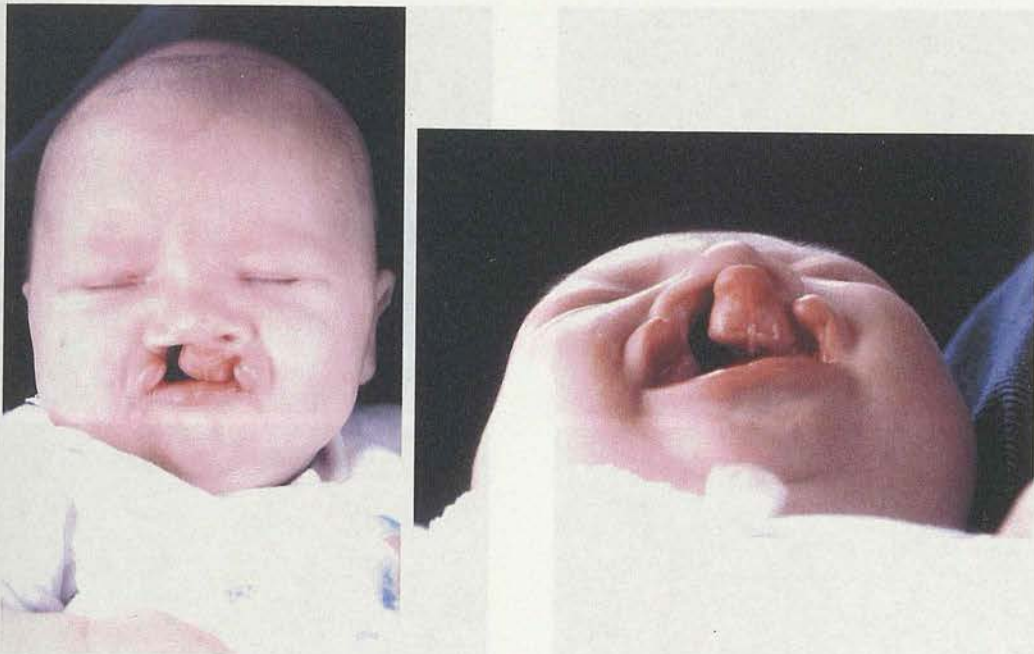


Fig. 1. Patient 1 at age 1 month: (left) anterior view and (right) worm's-eye view.

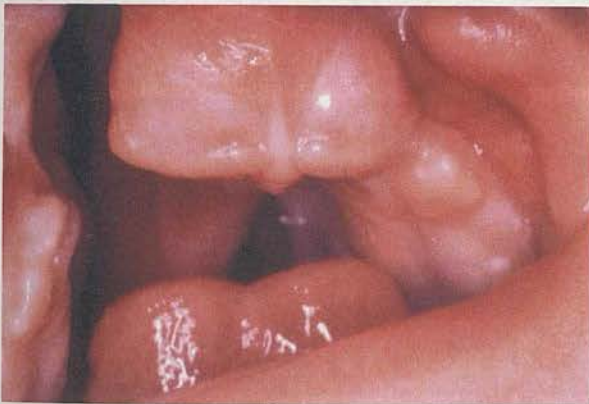


Fig. 2. Patient 1. Close-up view demonstrating the cleft.

First Year

The bilateral cleft lip repair was undertaken between 3 and 5 months of age. Presurgical orthopedics in the form of "cap and strap" was used in three of the 18 patients to attempt to reduce the protrusion of the premaxilla and prolabium in those cases where in the surgeon's opinion it facilitated muscle union.

The lip repair was achieved using the Millard rotation advancement technique. The prolabium was dissected free and the prolabial mucosa was used to fashion a sulcus. The muscle was mobilized widely and the lip was sutured in layers. Correction of the bilateral cleft nose was achieved through a partial intercartilaginous incision and mobilization of the

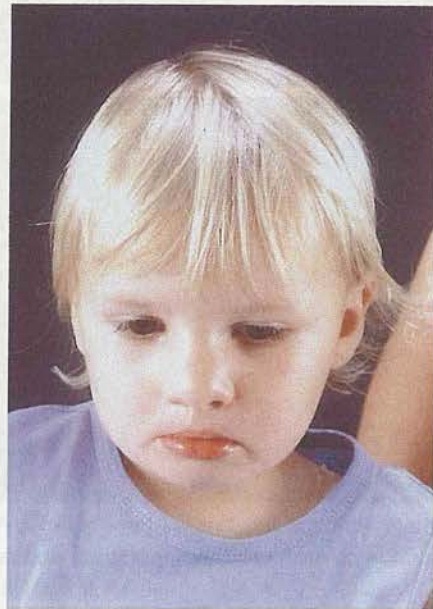


Fig. 3. Patient 1 at age 4 years.

alar cartilages and nostrils. The dislocated alar cartilages were repositioned and held by a temporary percutaneous nylon suture so that the domes were reapproximated and the caudal rotation overcome. The corrected intercartilaginous relationship was then secured with dissolvable sutures and the temporary nylon suture was removed. The nostrils are supported with Silastic Koken splints. At the same



Fig. 4. Patient 1 at age 14 years, before orthognathic surgery: (above) anterior view, (center) worm's-eye view, and (below) lateral view.

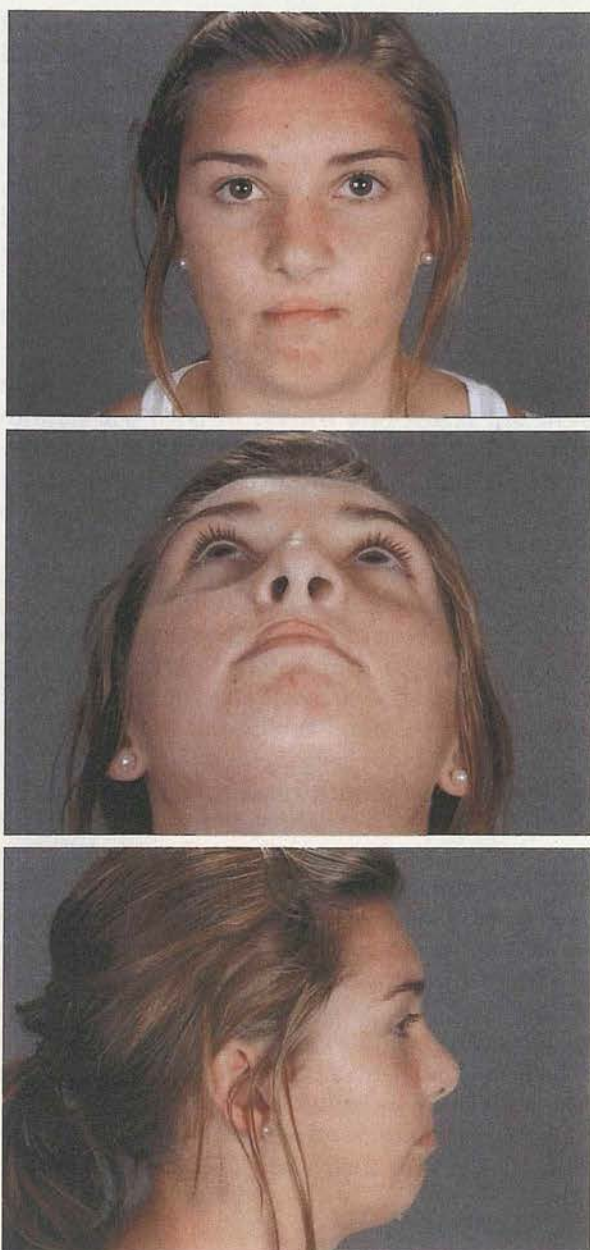


Fig. 5. Patient 1 at age 16 years following orthognathic surgery and rhinoplasty: (above) anterior view, (center) worm's-eye view, and (below) lateral view.

time as the repair of the lip, under the same anesthetic, the otolaryngologist examines the ears and inserts myringotomy tubes as indicated.

The patients are then reviewed at 1 week by the cleft surgeon and, under a short general anesthetic, the lip sutures are removed. They are then seen again 1 week later and then again at 6 weeks by the otolaryngologist if myringotomy tubes were inserted.

The cleft palate was ideally repaired at 6 months of age, using a modified two-flap palatoplasty with muscle identification, dissection, and repair. Anterior fistulas are left from the incisive foramen to the alveolus, to be repaired at the time of the alveolar bone graft. During the same anesthesia session, otologic examination is undertaken and myringotomy tubes inserted as necessary. Oral fluids can be commenced immediately after surgery and arm splints are worn on an as-needed basis.



Fig. 6. Patient 2 at age 4 months.

The patients are again seen 1 week postoperatively by the cleft surgeon and at 6 weeks by the otolaryngologist if myringotomy tubes have been inserted. The otolaryngologist will see the patient every 6 months until the ears are normal and myringotomy tubes are no longer required.

At 3 months after the palate repair, the speech pathologist reviews the patient's speech and language development. Review by the orthodontist is undertaken to outline the protocol management during childhood. A 1-year photograph is obtained.

Second through Fifth Years

Annual review by the cleft surgeon is undertaken. Nasendoscopy is performed at 5 years of age if indicated. Speech assessment is undertaken at 1.5, 2.5, 3.5, and 4.5 years of age. The cleft lip–cleft palate multidisciplinary meeting is attended at 1.5 and 4.5 years.

Sixth through Twelfth Years

Annual review by the cleft surgeon is performed and otolaryngology review is performed every 6 months if myringotomy tubes have been inserted; otherwise, review is performed every 12 months. The speech therapist reviews the patient at 8 and 12 years of age, and nasendoscopy is performed if indicated. Orthodontic review in conjunction with cephalometric radiographs is performed annually to monitor facial growth and the development of the oc-

clusion. The multidisciplinary team meeting is attended at ages 8 and 12 years.

Audiometry

Hearing levels of less than 20 dB are considered to be within the normal range, mild loss is between 20 and 30 dB, and significant loss is considered to be greater than 30 dB.

Orthodontic and Alveolar Bone Grafting

Assessment is performed at 4.5 years when primary dentition is complete. At 6 years of age, study models are used to record and assess the developing occlusion, and radiographs are used to assess the number, morphology, and position of the developing secondary dentition.

At approximately 8 to 9 years of age, the upper arch is expanded to align the upper incisors and to provide access for alveolar bone grafting. Rapid maxillary expansion is carried out over a 3- to 4-week period to establish the contour of the upper arch. Bone grafting to both alveolar clefts is performed at the same time. Cancellous bone is harvested from the iliac crest; bilateral gingival mucoperiosteal flaps are raised; lining flaps are formed back to the incisive foramen; a curved fragment of cortical bone is used to fashion the floor of the pyriform aperture and used as a buffer against which to pack the cancellous bone and the bone chips are packed into the alveolus, nostril floor, and anterior maxilla; and the graft is extended onto the lateral aspect of the pyriform aperture to support the alar bases on both sides. The bone graft is three-pronged, extending posteriorly to the incisive foramen, laterally onto the maxilla, and inferiorly to form the alveolus. At 13 years of age, orthodontic treatment is undertaken to align the dental arches and, if indicated, prepare them for orthognathic surgery.

Orthognathic Surgery

At approximately 17 or 18 years of age, final assessment of the facial pattern is carried out. Hand/wrist radiographs are used to help estimate that growth is complete, and lateral cephalograms and study models are used to help plan any proposed orthognathic surgery. The orthodontic appliances are used for control of the occlusion during surgery and retained to "fine tune" any minor occlusal discrepancies. Once they have had their orthognathic surgery, the patients are reassessed after a postoperative period of approximately 3 months with regard to the necessity of a forward sliding genioplasty to correct a recessive chin point. At this time, any deficiency of the philtral

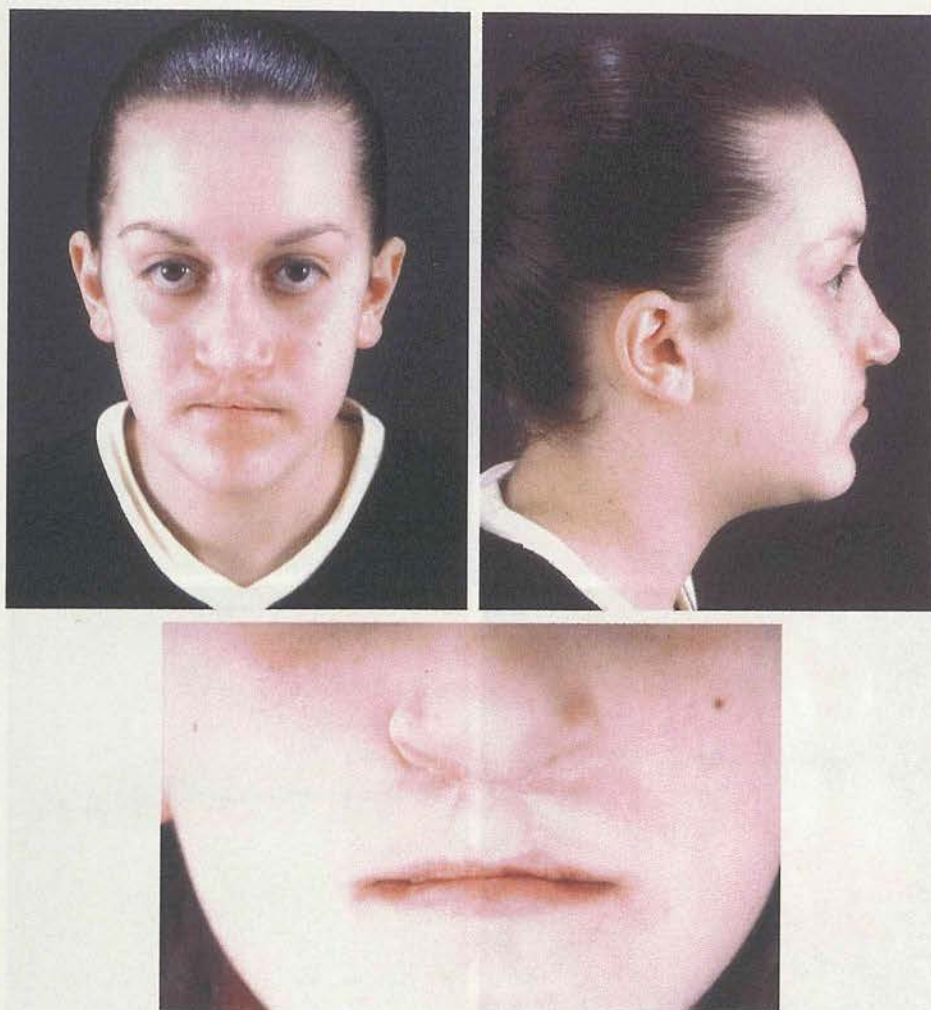


Fig. 7. Patient 2 at age 15 years, before Abbe flap: (above, left), anterior view, (above, right), lateral view, and (below) close-up.

area of the upper lip can be reconstructed with an Abbe flap from the lower lip. Once all this is complete, the nasal deformity in bilateral cleft patients is corrected with a rhinoplasty or septorhinoplasty.

Speech Evaluation

All assessments and reviews were conducted by two senior speech pathologists based in the Women's and Children's Hospital Craniofacial Unit with extensive experience in managing patients with cleft lip–cleft palate. Reliability was established by comparing ratings from recorded speech samples made during a patient's scheduled reviews in accordance with the Unit's protocol.

Speech was evaluated on the parameters of articulation, resonance, velopharyngeal competence, and intelligibility. The speech outcome at skeletal maturity was given a rating in accordance

with a revised Great Ormond Street Speech Pathology Assessment and Universal Parameters Ratings for reporting speech outcomes in cleft palate¹ as follows: articulation (consonant production errors), resonance (hypernasality, hyponasality), and nasal air emission (Table 1). A speech intelligibility rating was assigned to all patients to report the final speech outcome in this study as an indicator of the effectiveness of daily communication.

Nasal emission was evaluated by mirror misting testing and rated according to consistency and audibility. Nasendoscopy with lateral videofluoroscopy was performed in those cases of suspected or actual velopharyngeal incompetence. Dynamic testing was carried out using a split-screen simultaneous view with lateral videofluoroscopy and a rigid Storz 4-mm nasendoscope (Karl Storz, Tuttlingen, Germany), as described previously.²



Fig. 8. Patient 2 at age 23 years: (above, left) anterior, (above, right) lateral, (below, left) close-up, and (below, right) occlusion views are shown.

RESULTS

During the 32-year period from 1974 to 2006, the senior author (D.J.D.) completed the Unit's multidisciplinary treatment protocol from birth to maturity on a total of 19 patients with bilateral cleft lip–cleft palate. The age range at completion of their protocol of treatment was 16 to 32 years, and there were 12 male and seven female patients. The cleft lips were repaired between 3 and 12 months of age. The vast majority of patients ($n = 17$) had their lip repaired at approximately 4 months of age. One had the lip repaired at 12 months, as he had undergone multiple previous failed lip adhesion procedures before being referred to the Australian Craniofacial Unit. The mean age of bilateral cleft lip repair was 4.2 months. The cleft palates were repaired between 7 and 16 months, with a mean age at repair of 12 months.

The vast majority of patients, 79 percent (15 of 19), not surprisingly, had an anterior palatal fistula. This was usually corrected at the time of alveolar bone grafting.

All of the patients underwent alveolar bone grafting, with only one patient requiring a repeated bone graft to achieve a satisfactory result. The alveolar bone grafting was carried out between 8 and 18 years of age, with a mean age of 10 years. One patient had the alveolar bone graft at 18 years of age, and this timing was the patient's choice.

From an otolaryngologic point of view, only two patients did not require myringotomy tubes, with the remaining 17 patients requiring insertion on at least one occasion. Six of these patients needed repeated insertion of the myringotomy tubes because of extrusion or loss of the first set. All of the patients had hearing within the normal range on audiometry.

Table 1. Revised Great Ormond Street Speech Pathology Assessment and Universal Parameters Ratings

Assessment Tool	Description
Revised Great Ormond Street Speech Pathology Assessment	
Articulation	
CTCs	
0	Normal consonants
0–1	No CTCs
1	Anterior oral CTCs: dentalization, lateralization, palatalization, double articulation
2	Posterior oral CTCs: backing to velar, backing to uvula
3	Nonoral CTCs: pharyngeal articulation, glottal articulation
4	Passive CTCs: weak/nasalized/absent pressure consonants, nasal realizations
5	Developmental/phonologic errors
6	Other misarticulations
Universal Parameters Ratings	
Resonance	
Hypernasality	
0	Normal tone
1	Mild
2	Moderate
3	Severe
Hyponasality	
0	Absent/within normal limits
1	Present
Nasal air emission and/or nasal turbulence	
0	None/within normal limits
1	Present
Intelligibility	
0	Within normal limits: speech is always easy to understand
1	Mild: speech is occasionally hard to understand
2	Moderate: speech is often hard to understand
3	Severe: speech is hard to understand most or all of the time

CTCs, cleft type characteristics.

Speech

The speech outcomes are summarized in Table 2. Fourteen of the 19 cases (74 percent) were judged to be within normal limits at skeletal maturity, with the remaining five cases having mild difficulties only with speech intelligibility. Some of these cases judged to be within normal limits still had persistent but minor articulation and resonance distortions.

Speech therapy was recommended for nine of the 19 patients at some time during their management for a range of speech anomalies. The indications for therapy were articulation in seven cases, resonance/velopharyngeal insufficiency in four cases, and early childhood language delay in two cases. Two patients required intervention in more than one area. Speech outcomes will now be considered in more detail in terms of articulation, velopharyngeal insufficiency, and resonance.

Articulation

During the period of management, the presence of articulation errors was common, with 18 of the 19 patients affected at some stage during the course of their management. Most of these (15 of 19) presented with anterior cleft type character-

istics associated with their occlusion (i.e., dentalization, lateralization, and/or palatalization). Two cases had posterior and nonoral cleft type characteristics associated with velopharyngeal insufficiency (i.e., backing, pharyngeal, and/or glottal articulation). Three cases presented with developmental articulation substitutions, and two cases demonstrated labialization of fricatives in association with their class III occlusion. At skeletal maturity and completion of their treatment protocol, five patients were evaluated as having a mild articulatory disorder (anterior oral cleft type characteristics: dentalization, labialization, palatalization, and residual developmental articulation errors).

Resonance

Distortions were similarly common at some stage during development, with 15 of the 19 patients affected. Management of hypernasality included speech therapy, fistula closure, and pharyngoplasty/posterior pharyngeal wall implant. Management of hyponasality included septorhinoplasty. At completion of their treatment protocol, four patients were judged to have a grade 0 anomaly (normal resonance) and 15 had a grade

Table 2. Speech Outcomes*

Patient	Articulation CTCs	Resonance		Nasal Emission	Intelligibility	Maxillary Advancement with or without Fistula Closure	VPI after Orthognathic Surgery	Pharyngoplasty
		Hypernasality	Hyponasality					
1	1	1	0	1	0	Yes plus fistula	Yes	Yes (PPWI)
2	1	1	0	1	1	Yes	Yes	No
3	0	1	1	0	0	Yes	No	No
4	1	1	0	1	1	Yes	Yes	Yes (SBPF)
5	0	0	0	0	0	Yes plus fistula	Yes	Yes (IVV)
6	1	1	0	1	0	Yes plus fistula	Yes	No
7	1	0	0	0	0	Yes plus fistula	Yes	Yes (SBPF)
8	1	1	0	1	0	Yes	Yes	No
9	1	1	0	1	1	Yes plus fistula	No	Yes (PPWI)
10	5	1	0	0	0	Yes	No	No
11	1	1	0	1	1	Yes	Yes	Yes (SBPF)
12	0	2	0	0	0	No	N/A	No
13	1	0	1	0	0	No	N/A	No
14	3	1	0	1	1	Yes	No	Yes (SBPF)
15	1	1	1	1	0	Yes plus fistula	No	No
16	1	1	1	1	0	No	N/A	No
17	5	1	0	1	0	Yes	Yes	Yes (SBPF plus reoperation)
18	1	0	0	0	0	Yes	No	No
19	1	0	0	0	0	Fistula closure	N/A	No

CTCs, cleft type characteristics; VPI, velopharyngeal insufficiency; PPWI, posterior pharyngeal wall implant; SBPF, superiorly based pharyngeal flap; IVV, intravelar veloplasty; N/A, not applicable.
 *The descriptions of the ratings 1, 0, and 2 are as described in Table 1.

1 (mild) anomaly (hypernasality, *n* = 11; mixed nasality, *n* = 3; and hyponasality, *n* = 1).

Velopharyngeal Insufficiency

Audible nasal air emission and nasal turbulence was also common during childhood. It occurred in 14 of 19 cases at some point during development (five within normal limits, 12 mild, and two moderate). Management included speech therapy, fistula closure, and pharyngoplasty. Eight of 19 patients underwent surgery for speech (superiorly based flap, Orticochea, intravelar veloplasty, or augmentation of the posterior pharyngeal wall with an implant).³

In five of these patients, the velopharyngeal incompetence occurred after the Le Fort I maxillary advancement orthognathic surgery. The final outcome after surgery for speech was that 14 cases achieved speech judged to be within normal limits, whereas five had mild problems with speech intelligibility (Table 2).

Orthodontics. A class III malocclusion was present in 17 of the 19 patients, and the remaining two patients had a class I occlusion (Table 3). Of those with a class III malocclusion, 14 underwent orthognathic surgery and the remaining three patients declined the offer of surgery. Bimaxillary surgery was performed on six patients (Le Fort I maxillary advancement and sagittal split mandibular setback), and eight patients underwent maxillary advancement alone. Simultaneous augmen-

tation of the hypoplastic midface region with onlay bone grafts taken from the inner table of the iliac crest was performed in 13 of the 14 orthognathic surgery patients.

Final Surgery. Nearly all patients (17 of 19) required a rhinoplasty to correct the significant nasal deformity associated with these bilateral cleft patients. In all but one case, this was accomplished by means of an open approach. Simultaneous correction of the deviated septum (i.e., septorhinoplasty) was performed in 11 of the 17 rhinoplasty cases.

Upper lip augmentation was necessary in a total of eight cases. An Abbe flap was used to reconstruct the deficient philtral region and to allow a degree of lengthening of the columella, which was used to give a more appropriate nasal tip projection (Figs. 6 through 8).

Overall revision surgery in the form of, for example, scar revision or tidying up of the nose or lip was performed with between one and five procedures, with a mean of 2.3 procedures per patient. After completion of their orthognathic surgery, three patients had a forward sliding genioplasty to complete the improvement in their facial profile (Table 4 and Figs. 2 through 5).

Dental. All patients had varying degrees of missing upper teeth. In the majority of cases, this was addressed with partial upper dentures, but five patients were either waiting for, or had fitted, osseointegrated implant retained dentures.

Table 3. Maxillary and Mandibular Positions at Skeletal Maturity

Patient	SNA (Degrees)	SNB (Degrees)	ANB (Degrees)	Occlusion Class	Orthognathic Surgery	Onlay Bone Graft
1	68	72	-4	III	Maxillary advancement	Yes
2	73	76	-3	III	Bimaxillary	Yes
3	83	83	0	III	Bimaxillary	Yes
4	66	69	-3	III	Bimaxillary	Yes
5	78	74	4	III	Bimaxillary	Yes
6	75	71	4	III	Maxillary advancement	Yes
7	80	80	0	III	Maxillary advancement	Yes
8	80	82	-2	III	Maxillary advancement	Yes
9	77	71	6	III	Maxillary advancement	Yes
10	86	82	4	III	Maxillary advancement	Yes
11	69	70	-1	III	No	Yes
12	78	78	0	I	No	Yes
13	66	68	-2	I	No	Yes
14	87	92	-5	III	Bimaxillary	Yes
15	71	73	-2	III	Maxillary advancement	Yes
16	75	79	-4	III	No	No
17	78	70	8	III	Maxillary advancement	Yes
18	67	75	-8	III	Bimaxillary	Yes
19	75	73	2	III	No	No

SNA, sella, nasion, point A angle; SNB, sella, nasion, point B angle; ANB, SNA - SNB.

Table 4. Secondary Surgery*

Patient	Genioplasty	Septorhinoplasty/Rhinoplasty	Abbe Flap to Upper Lip	No. of Revisions
1	No	Septorhinoplasty	No	4
2	No	Rhinoplasty	No	1
3	No	No	No	1
4	No	Septorhinoplasty	No	2
5	Yes	No	Yes	3
6	No	Septorhinoplasty	No	2
7	No	Rhinoplasty	No	1
8	No	Septorhinoplasty	No	2
9	Yes	Septorhinoplasty	Yes	2
10	No	Rhinoplasty	No	3
11	No	Rhinoplasty	No	1
12	No	Rhinoplasty	No	2
13	No	Septorhinoplasty	No	2
14	No	Septorhinoplasty	No	3
15	No	Septorhinoplasty	No	2
16	No	Septorhinoplasty	No	5
17	Yes	Septorhinoplasty	Yes	3
18	No	Septorhinoplasty	No	3
19	No	Septorhinoplasty	No	1

*Genioplasty, rhinoplasty, and Abbe flap.

The treatment protocol is exemplified by the photographs of a typical case shown in Figure 1. This patient demonstrates the treatment from birth with a bilateral cleft palate to skeletal maturity. She required correction of the bilateral cleft lip at 4 months of age and repair of the palate at 11 months of age. Alveolar bone grafting was performed, and she required a superiorly based pharyngoplasty. Speech was recorded as normal at completion of her treatment protocol. She had a Le Fort I maxillary advancement, onlay bone grafts to the cheeks, and subsequent lip revision and open septorhinoplasty to complete her surgery.

DISCUSSION

The patient with a bilateral cleft–cleft palate has a much more complex deformity to correct compared with the unilateral cleft. The challenge to the surgeon and the cleft team is not only to restore the form and function of the lip, nose, and palate, but (critically) to remove the stigma of the cleft.

The cleft lip and primary nose deformity were repaired at the same time at a mean age of 4.2 months, and the technique was consistent for all the patients in the study. The palate was repaired at a mean age of 12 months using the two-flap palatoplasty technique. All patients with complete clefts had an anterior fistula. However, this was corrected

at the time of alveolar bone grafting. Despite the large bony defect to reconstruct, only one patient required two separate bone grafting procedures to fill the defect. There is significant maxillary hypoplasia in patients with bilateral cleft lip–cleft palate, and this is reflected in the relatively high percentage of patients undergoing orthognathic surgery to correct the dentoskeletal discrepancy. Seventy-three percent of patients underwent orthognathic surgery. This compares with 30 percent in the previous studies by the senior author⁴ in the unilateral cleft lip–cleft palate group and 13 percent in the isolated cleft palate group.⁵ This is understandable, as the tissue deficit in bilateral clefts is greater than in the other two groups.

Overall, evaluating the speech outcomes of all 19 cases with bilateral cleft lip–cleft palate, although the majority were judged to be within normal limits, many still had mild articulation errors. These commonly featured anterior oral cleft type characteristics (i.e., dentalization, lateralization, and/or palatalization). Midface advancement osteotomies at skeletal maturity were performed in 14 cases, which is higher than the unilateral cleft lip–cleft palate cases or the isolated cleft palate cases but reflects the Unit's philosophy of removing the stigmata of the cleft. Of the eight patients who required pharyngoplasty, five had the velopharyngeal incompetence treated by means of Le Fort I maxillary advancement orthognathic surgery. The osteotomy rate is relatively high because the goal of management is full function and removal of the stigma of the cleft. The authors believe that this is the appropriate goal. If orthognathic surgery is required to achieve this, it should be done, so osteotomy rates should not be used as a measure of the success of the cleft program.

An anterior palatal fistula was present in 15 of 19 cases. Surgical closure of fistulas in eight patients resulted in improved speech intelligibility (reduced nasal emission and hypernasality, with stronger high-pressure consonant profile).

The Abbe flap for correction of the secondary lip deformity provided a very satisfactory cosmetic result. The upper lip requiring an Abbe flap often had a combination of a central vermilion deficiency, short vertical height, scarring, and a short prolabium. The Abbe flap was used in a total of eight patients (42 percent) to very good effect when combined with an open rhinoplasty, the prolabium being advanced into the columella and the cross-lip flap filling the residual defect (Figs. 6 through 8).

The rationale for sparing the alveolar and post-alveolar region in the early cleft repair is that it leaves a virgin field for the alveolar bone graft, which the authors see as a vital step in bringing about full dentofacial function, speech function, and harmo-

nious facial aesthetics. The tradeoff is the presence of the postalveolar fistulas for a number of years. The discussions about the worth of different cleft protocols abound with arguments around the compromises that are made vis-à-vis speech versus facial growth. What is clear from this and the preceding birth-to-maturity studies from the Australian Craniofacial Unit is that function can be maintained/restored and the stigma of the cleft removed; however, the burden of treatment for the patient with bilateral cleft lip–cleft palate is significantly increased.

CONCLUSIONS

The patients in this study have all been managed by a single surgeon (D.J.D.) and have followed a strict protocol management under the guidance of a multidisciplinary cleft lip–cleft palate team. This cohort of patients is continually increasing in size as further cases reach maturity.

We believe that the treatment protocol at the Australian Craniofacial Unit is effective and helps patients with bilateral cleft lip–cleft palate to become normal functioning members of society by having the twin objectives of full restoration of function and removal of the stigma of the cleft. However, in bilateral cleft lip–cleft palate, there can be little doubt that these remain challenging cases in which to achieve our management goals.

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PATIENT CONSENT

Patients provided written consent for the use of their images.

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Mandibular Lengthening by Distraction for Airway Obstruction in Treacher-Collins Syndrome: The Long-Term Results

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Mandibular lengthening by distraction was performed in a 6-year-old severely affected Treacher-Collins syndrome patient who was tracheostomy dependent. As previously reported, this procedure permitted tracheostomy removal once distraction was complete. Now that the patient is skeletally mature, the long-term results of this intervention are reported with regard to his clinical outcome and an assessment of the anatomical changes in the upper airway during growth. Although the distraction could be considered a success in that it enabled permanent decannulation and improved the minimum cross-sectional area of the upper airway, there was no further increase in the minimum cross-sectional area of the upper airway during childhood growth. It is significant that the abnormal growth pattern of the mandible, which is characteristic of this syndrome, did not alter from its preoperative pattern once distraction was completed.

increase in mandibular length and the resulting change in profile are shown (Fig 2).

Key Words: Airway obstruction, mandibular lengthening, Treacher-Collins syndrome

CASE REPORT

A 6-year-old white boy with severe Treacher-Collins syndrome, who had been tracheostomy dependent since the age of 10 months (Fig 1), underwent bilateral mandibular distraction osteogenesis. This was done using bilateral external devices. Distraction of 1 mm/d was undertaken for 20 days, followed by 6 weeks of consolidation. The immediate

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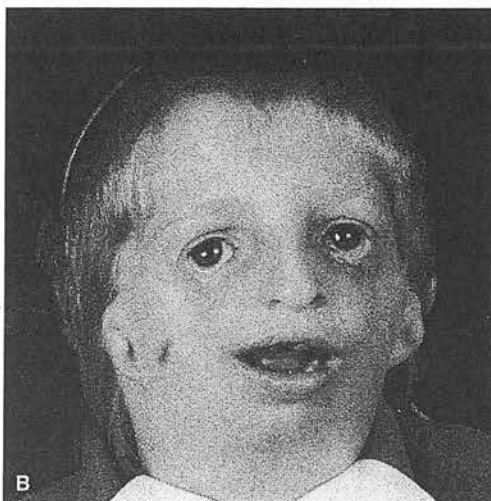


Fig 1 Frontal and lateral views of the patient before distraction at the age of 6 years, with his tracheostomy in situ.



Fig 2 Frontal and lateral views of the patient after distraction at the age of 7 years.

The patient then underwent investigation after trial occlusion of his tracheostomy with oximetry and formal sleep studies. These were satisfactory, and he subsequently underwent formal decannulation as we have previously reported.¹

Our initial report¹ identified that he maintained a safe airway for 18 months. Shortly after this time, however, he gradually developed signs of sleep apnea and upper airway obstruction, which was confirmed by polysomnography when investigated.

This has been managed by a continuous positive-pressure airway mask for nighttime use. The patient has not required any other form of intervention to improve his airway management, however. He has been kept under regular review, and serial computed tomography (CT) scans have been used to

monitor his facial growth, especially upper airway and mandibular growth.

He has now reached skeletal maturity (Fig 3). Definitive orthognathic surgery in the form of bimaxillary surgery is planned once preparatory orthodontics have been completed.

DISCUSSION

Treacher-Collins syndrome is a clefting condition in which upper airway anomalies, including pharyngeal hypoplasia, are well recognized.^{2,3} Because of the micrognathia that is a feature of severely affected individuals with the syndrome, there is a high risk of sleep apnea,⁴ which has been reported to be as high as 25%.⁵ The potential for using distraction to improve the upper airway has been recog-

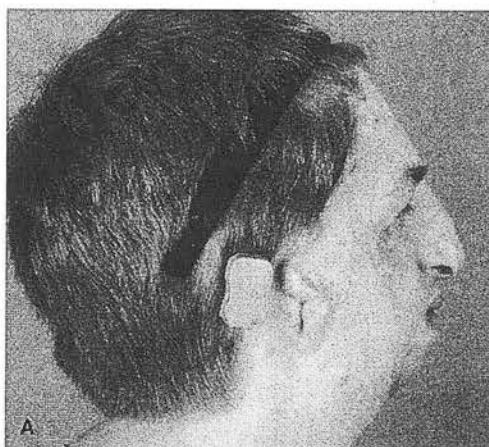


Fig 3 Frontal and lateral views of the patient at the age of 17 years.

nized since distraction of the craniofacial skeleton was first reported.⁶

Our initial report highlighted the fact that the airway improved after distraction to the extent that the patient could be decannulated. This initial clinical improvement has also been reported by other authors in cases of Treacher-Collins syndrome^{7,8} as well as Nager syndrome.⁸

Although others have attempted to measure the airway before and after distraction surgery in Treacher-Collins syndrome,⁹ we prefer to measure the airway using the reconstructed images from three-dimensional CT scans. Detailed three-dimensional CT scans were undertaken immediately before and after distraction and a further four times (up to 10 years) after surgery. These studies have enabled investigation of the upper airway in three dimensions and assessment of the results of the distraction process, which are shown in two dimensions using the midline sagittal cut in serial CT scans (Fig 4).

These results have been analyzed with respect to the critical value of the minimum cross-sectional area of the upper airway and have been compared with normal patient data. The level at which the minimum cross-sectional area occurred in the upper airway was not constant but changed during development. The results show that the minimum cross-sectional airway increased during the period of distraction but without reaching the age-adjusted normal value (Fig 5). The subsequent serial scans demonstrate that the minimal cross-sectional airway did not increase further during normal childhood growth.

Similarly, measurements of the cross-sectional

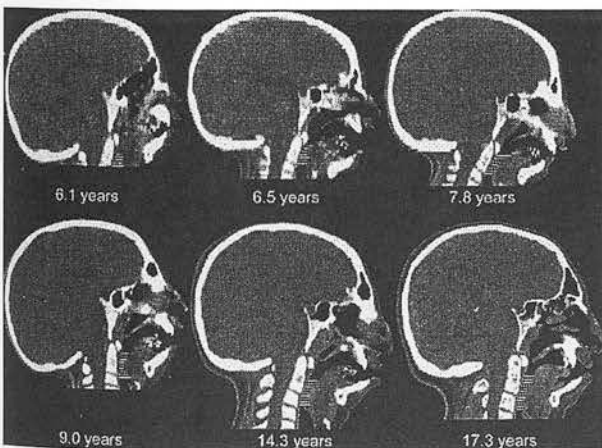


Fig 4 Midline sagittal cuts of serial computed tomography scans demonstrating the changes in the upper airway before distraction and with subsequent growth.

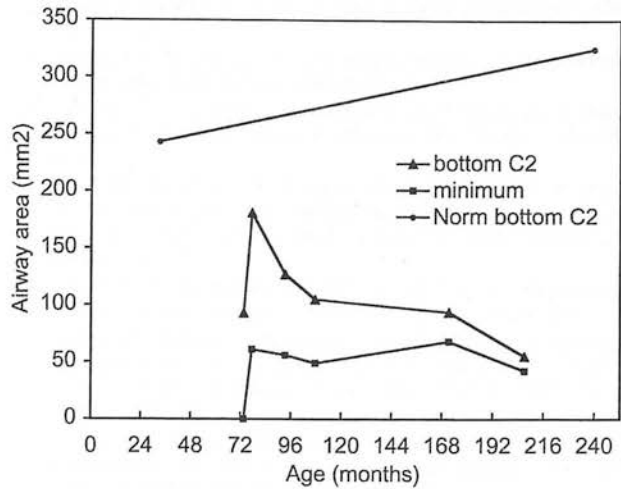


Fig 5 Diagram shows the changes in the minimum cross-sectional upper airway. Although this increased during the distraction procedure, it has undergone little change since completion of distraction. The cross-sectional area of the airway at a fixed point (bottom of C2 vertebral body) is also shown and compared with normal data.

area at a fixed point of the upper airway (base of C2 vertebral body) also demonstrated that there was no increase at this level in the cross-sectional area during growth. This longitudinal study clearly demonstrates that skeletal growth fails to increase the cross-sectional area of the upper airway. We suggest that this is because the underlying pathology of Treacher-Collins syndrome is that of a clefting condition, which has inherent impaired growth potential, and this impaired growth potential remains unaltered by skeletal distraction.

Further detailed study of the pattern of mandibular growth after distraction was undertaken using wire frame reconstructions to study the growth pattern in three dimensions. Wire frame studies of the three-dimensional CT scans of the mandible and skull base were performed using the Persona software package developed in the Australian Craniofacial Unit.¹⁰ These reconstructions have shown that mandibular growth after distraction continued in the same pattern as before the intervention, with an abnormally high mandibular plane angle, which is characteristic of Treacher-Collins syndrome. This combination of an increasingly high mandibular plane angle and the failure of distraction to produce a long-term change in the growth pattern of the mandible in Treacher-Collins syndrome creates the skeletal basis for ongoing upper airway obstruction.

In conclusion, this case demonstrates that the intervention using mandibular distraction has en-

abled the airway to be improved such that the tracheostomy has remained closed during the remainder of childhood. The initial improvement in the measured minimum upper airway that occurred during distraction has not continued during skeletal growth, however, but has remained static. The clinical consequences were the re-emergence of the symptoms of sleep apnea, which required treatment with a continuous positive-pressure airway mask at night.

This case report describes the long-term results of a single episode of mandibular distraction undertaken for upper airway management in a case of severe Treacher-Collins syndrome. The exact role for mandibular distraction in our treatment protocol for Treacher-Collins syndrome will be determined by additional long-term outcomes from larger studies.

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Treacher Collins Syndrome: Protocol Management From Birth to Maturity

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Background: Management of patients with Treacher Collins syndrome is complicated and involves multiple disciplines working in concert to achieve a common outcome. This article reviews the experience at the Australian Craniofacial Unit and describes the protocol for management.

Methods: Fifty patients were treated during the last 30 years. The records of these patients were reviewed to establish what interventions they required and how these fit into a protocol for management.

Results: The protocol for management of Treacher Collins syndrome can be divided into 3 epochs. In the first epoch from birth to age 2, airway and feeding problems were the main focus. Four patients required tracheostomy. Of these, 1 died and the others received mandibular distraction. Hearing is evaluated and addressed early. Eleven patients (23%) required repair of a cleft palate. In the second epoch (aged 2–12 y), speech therapy is critical as is a focus on integrating into the education system. During this epoch, reconstruction of the upper face was performed either with bone grafts or with vascularized bone flaps. Both required repeat bone grafts later. In the third epoch (aged 13–18 y), orthognathic surgery was performed. Revision surgery and further bone grafting were performed again at around age 18. Patients reported being generally happy with their appearance and with few exceptions were able to complete education, gain employment, and feel socially accepted.

Conclusions: Management of patients with Treacher Collins syndrome should be through a multidisciplinary protocol to achieve good results while minimizing confusion and unnecessary surgery.

Key Words: Treacher Collins, protocol management, multidisciplinary

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Treacher Collins syndrome describes a complicated assortment of craniofacial malformations resulting in problems affecting the form and function of the eyes, ears, nose, maxilla, palate, mandible, and airway. Although first described in 1889 by Berry,¹ Treacher Collins's² later description in 1900 ultimately resulted in his eponymous association with the syndrome. The scientific nomenclature most commonly used is mandibulofacial dysostosis, although this is not specific to Treacher Collins syndrome.

As a result of the complex assortment of deformities, it is deemed essential that each patient receives comprehensive multidisciplinary care for optimal outcomes. Because the incidence of Treacher Collins is only 1 in 50,000 live births,³ much of the care of these patients is documented sporadically as clinical reports or descriptions of operative interventions by the various specialties involved. Therefore, the purpose of this review was to examine the management of patients with Treacher Collins syndrome during a 30-year period from a broad perspective to determine what successes and failures occur along the way. Finally, this article will describe how best to organize the multiple disciplines involved and present a protocol for future management and assessment of outcomes.

METHODS

Fifty patients were registered as patients at the Australian Craniofacial Unit in Adelaide from 1975 to 2005. All patients had a confirmed diagnosis of Treacher Collins syndrome by the craniofacial team and clinical genetics. Patients were not included in the study if the diagnosis was uncertain. In addition, patients who were seen but did not have adequate records were excluded. Patient records were examined for clinical features, interventions, and outcomes.

RESULTS

Overall, 50 patients were treated during the period reviewed. Three of these patients had incomplete records and were not included in the study. Of the 47 included in the study, 23 were males and 24 were females. Fourteen patients were treated from birth to maturity; the remainder of the patients were evaluated and treated either at a later stage of childhood or as adults. One patient died of septicemia at the age of 1 year. This patient was a severely affected individual and had undergone tracheostomy but had received no other surgical treatment. Another patient died in adolescence in a vehicle crash, making the syndrome-related childhood mortality 1 of 47. Eight families had Treacher Collins syndrome, making up 20 of the patients seen, whereas 27 were new mutations.

The physical features and problems found in this patient group are listed in Table 1 and are generally consistent with other reports.³ The microform of Treacher Collins is often difficult to detect. In several of the families in our series, it was not evident that 1 of the parents was affected until a second affected child was born. Based on our experience, the subtle evidence of deformity in the microform state is most evident around the zygoma with very mild

TABLE 1. Features of Treacher Collins Syndrome and Incidence Noted in this Series of Patients

Feature	n	%
Downslanting palpebral fissures	47	100
Malar hypoplasia	44	94
Mandibular hypoplasia	42	89
Hearing disability	42	89
Malocclusion	41	87
Auricular deformity	40	85
Middle ear deformity	40	85
Speech problems	34	74
External auditory canal deformity	30	64
Absent eyelashes	29	62
Lacrimal deformity	27	57
Airway compromise at birth	22	47
Epiphora	20	43
Lower eyelid coloboma	20	43
Visual disability	16	34
Palatopharyngeal incompetence	14	30
Pierre Robin sequence	14	30
Cleft palate	11	23
Otitis media requiring tubes	9	19
Obstructive sleep apnea beyond childhood	9	19
Ptosis	7	15
Choanal atresia	5	11
Macrostomia	5	11
Ear tags	4	9
Cleft lip	1	2
Inner ear deformity	0	0

downslanting palpebral fissures and depressions in the zygomatic body (seen in the older patient in Fig. 1).

Airway Management

Previously, the airway management strategy used by this unit for retrognathism was published.⁴ The focus is on managing the airway with positioning, nasopharyngeal airway, or positive pressure masks, using tracheostomy only as a last resort. No neonatal distraction or tongue-lip adhesions were performed. In this series, 4 patients required tracheostomy during infancy. One patient died at 1 year from septicemia related to a pulmonary infection and was the only patient to die in this series from a disease-related problem. Three were decannulated after mandibular distraction osteogenesis had been performed (Table 2). Two other patients required emergency tracheostomies later in childhood. One was a result of food aspiration; and the other, an inability to intubate for elective surgery by a different hospital that did not routinely care for children with craniofacial deformities.

Airway problems can extend into childhood as well. Ten patients were found to have obstructive sleep apnea during their childhood years. Of these patients, 2 had no previous airway problems. Tonsillectomy and adenoidectomy was performed in 7 patients, and 4 patients required continuous positive airway pressure (CPAP) beyond age 10. Bimaxillary advancement performed during the teenage years resolved the problem for 2 of the 4 patients on CPAP, whereas the other 2 are still awaiting surgery.

Genetics

Only 4 of the patients treated at this unit had formal genetic testing because the diagnosis is usually clinically obvious and because of cost constraints. Of these 4, 2 were confirmed to have a mutation in the *TCOF1* gene. In all, 57% of the patients were considered new mutations, whereas 43% were familial.

Ophthalmology

Patients with Treacher Collins syndrome experience a variety of periorbital and ophthalmological problems. In this series, downslanting palpebral fissures were noted in all 47 patients. Other deformities in decreasing incidence included absent medial lower lid eyelashes, lower lid lacrimal deformity, epiphora, lower lid coloboma, vision impairment requiring treatment, and ptosis. Although cataracts have been described in the literature,⁵ none were found in this series.

Absence of the lower lacrimal puncta lead to documented epiphora in 20 patients. No patient with epiphora required treatment other than routine eye care.

Ophthalmological problems included astigmatism, hypermetropia, and squint. Overall, 16 of the patients treated required either corrective lenses or surgery to improve their vision. Three patients were noted to have amblyopia.

Ears/Hearing

Almost all of the patients in this series had a conductive hearing loss with only 1 patient having a mixed hearing loss. All patients were screened for hearing in infancy and then again during childhood when more formalized testing could be done. The hearing loss was categorized into normal (<20 dB), mild (20–35 dB), moderate (35–50 dB), and severe (>50 dB) by audiometry that was usually performed at age 3 and again around age 8. Of the 46 patients who were tested for hearing, the distribution was as follows: normal (5 patients), mild (5 patients), moderate (6 patients), and severe (30 patients). All patients with hearing loss were given bone-conduction hearing aids.

Nine patients required tympanostomy tubes including 1 of the 11 patients with a palatal cleft. The remainder of the patients with cleft palate did not require tubes. One patient had a middle ear reconstruction with a ceramic ossicular chain. She was reported as having 15-dB gain but continued to require hearing aids.

External ear appearance was classified using the same auricular deformity classification previously published for hemifacial microsomia.⁶ Under this system, a normal ear is labeled A0, a small ear with malformation retaining the characteristic features is labeled A1, an ear with a rudimentary helix is labeled A2, and an absent pinna with a malformed lobule is labeled A3. Auricular deformity was present in 87% of the patients. The degree of hearing impairment was noted to correlate with external ear deformity as noted in Figure 2.

Speech

Speech abnormalities affected 34 of 46 patients evaluated. Typical speech problems included abnormal resonance dominated by hyponasality attributed to the size restriction in the nasal passages and oropharynx as well as hearing loss. Hyponasality was also noted in association with those children who had a cleft palate and velopharyngeal insufficiency. Articulation errors were found in 17 of 46 patients and were attributed to the malocclusion with anterior open bite and retroglossa.

Treatment consisted of review by an audiologist and an otolaryngologist within the first year to evaluate and correct hearing loss where possible. After this, speech and language therapy was initiated as soon as possible. Particular attention was given to maladaptive patterns related to the abnormal oral anatomy. When



FIGURE 1. From left to right, 3 generations of Treacher Collins with increasing severity. The only feature noted in the eldest patient was the orbitozygomatic depression.

these patterns developed, the children were given exercises to correct these patterns and ultimately prepare them for orthodontic and orthognathic correction. All patients were able to achieve excellent speech results except one who was from an underdeveloped nation and had a severe hearing loss and was not treated until adulthood.

The incidence of cleft palate was 11 of 46 patients in this series. Patients who had a cleft palate were be unable to have a palatal repair at the typical time (<12 mo) because of the complicated airway management. The average age at palate repair in this group was 2.1 years with only 1 patient having a cleft palate repair before 1 year of age.

Early attention to feeding is also an important issue. Twenty-two of the patients in this series were noted to have some degree of feeding difficulty, which was attributed to respiratory problems. Nine patients required either nasogastric or gastrostomy feeding during the first year of life of whom 8 had cleft palates. In patients with the most severe conditions, there were also some problems with oral aversion or hypersensitivity.

Psychosocial

On psychologic testing, 2 of 46 patients evaluated were noted to have a nonverbal intellectual disability. Most of the patients who had severe hearing loss also had trouble on the verbal examination, but this was not considered a true intellectual disability but rather a function of the hearing loss. It was part of the protocol to have each child evaluated by the psychologist before starting school and then at least once again several years into school. Formal testing usually consisted of the Wechsler Intelligence Scale for Children or the Griffith Mental Development Scale.

Of the 14 patients who were treated in this unit from birth to maturity, 12 had successful social outcomes. They completed education to year 12 and either have pursued further education/technical training or have begun employment. Of the 2 who did not achieve these goals, one had multiple medical problems and intellectual disabilities, whereas the other has had ongoing social/legal problems. Only 1 patient of the 14 described teasing as a significant problem during childhood that was difficult to overcome.

Facial Reconstruction

In all, 33 of 46 patients underwent orbitozygomatic reconstruction. Before 1986, the primary method of reconstruction was on-lay bone grafts to the deficient zygomatic area. Seven patients were treated during this period, 2 of whom also had orbital osteotomies to advance the zygoma. One patient during this period received a vascularized bone flap based on the temporalis muscle. Between 1986 and 2000, 15 patients received the vascularized bone flap procedure and only 5 underwent on-lay bone grafting alone, all of whom were adults at the time of their primary reconstruction. Finally, in patients treated after 2000, 5 were treated with on-lay bone grafting and only 1 patient received a vascularized bone graft.

TABLE 2. Airway Outcomes for Patients Who Required Tracheostomy in Infancy

Patient No.	Decannulation	Details
1	No	Died at age 1 y from sepsis
2	3 y	Distraction: 13-mm advancement
3	1 y	Distraction: 15-mm advancement
4	6 y	Distraction: 25-mm advancement

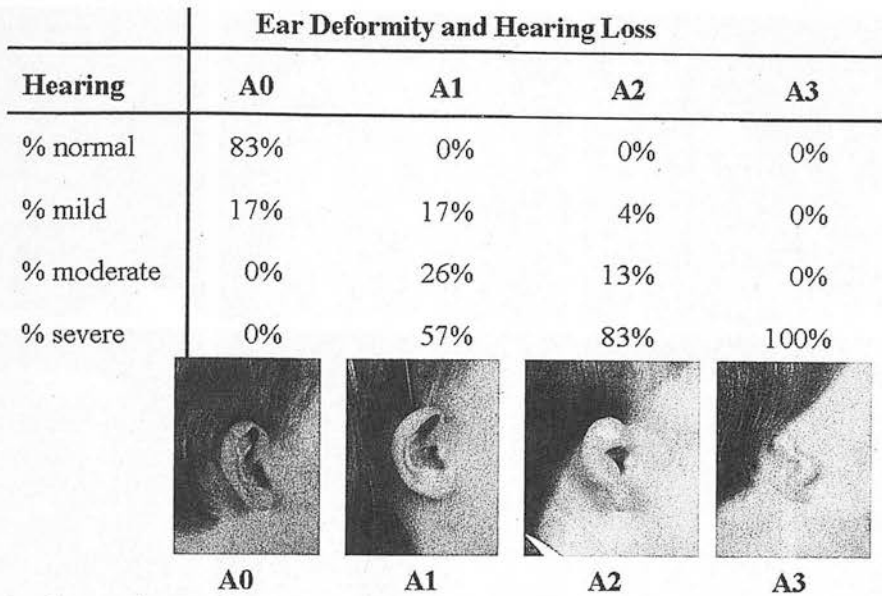


FIGURE 2. The severity of external ear malformation correlated with hearing loss.

Ten patients were followed from birth to maturity in this unit, and their results are summarized in Table 3. In two-thirds of these patients, a recurrent deformity was noted as seen in Figure 3. This required additional bone grafting and occurred whether the initial procedure was a vascularized bone flap or bone graft.

Treatment for the eyelid deformity consisted of musculocutaneous transposition flaps from the upper eyelid to the lower lid in 28 patients. A lateral canthopexy was also performed to create a normal palpebral slant. This was done at the same time as the initial orbitozygomatic bone grafting procedure.

Surgical reconstruction of deformed ears depended on the severity. Of the 20 patients with either A0 or A1 ears, 5 underwent an otoplasty procedure to correct the overhanging helix and reduce ear prominence. Three of the 26 patients who had type A2 or A3 ears underwent a staged reconstruction using rib cartilage at age 6, and 1 patient had an osseointegrated prosthetic ear reconstruction, which was performed in adulthood. The remainder of patients either chose no reconstruction or are awaiting reconstruction.

Orthodontic treatment and orthognathic surgery was performed once dental development was complete. Nine patients were

treated with orthodontics and did not require orthognathic surgery. Ten others underwent orthognathic surgery. Two of these patients were also treated to relieve upper airway obstruction. Before surgery, all patients had an anterior open bite, which was corrected with either a posterior impaction or an anterior opening Le Fort I. This was usually combined with mandibular surgery to correct the horizontal malocclusion (Table 4). After surgery, all patients had correction of the open bite. The 2 patients with sleep apnea no longer required CPAP after surgery.

Seventeen patients had a sliding osseous genioplasty, and 11 had a rhinoplasty. The rhinoplasty consisted of reducing the dorsal hump and adding tip projection with cartilage grafts when needed. In 6 of the patients who underwent rhinoplasty, a rib graft was used to reconstruct the nasal dorsum.

DISCUSSION

During the last 30 years, the Australian Craniofacial Unit has sought to organize and provide multidisciplinary care to patients with craniofacial deformities. Because patients with Treacher

TABLE 3. Initial Reconstructive Procedure and Subsequent Procedures in 10 Patients Who Had Reached Maturity at the Time of the Study

Patient No.	Year	Age, y	Initial Orbitozygomatic Reconstruction	Outcome
1	1980	4	Iliac crest graft, orbital osteotomies	Vascularized bone flap at age 10, bone graft at age 21
2	1983	5	Vascularized calvarial bone flap	Bone graft performed at age 14
3	1983	4	Calvarial graft	No additional procedures
4	1984	4	Calvarial and rib graft	Repeated bone graft at age 6, 13, 18, and 20
5	1986	7	Vascularized calvarial bone flap	Bone graft at age 16
6	1988	4	Vascularized calvarial bone flap	Required bone grafting at age 19
7	1989	6	Vascularized calvarial bone flap	Required bone grafting at age 13, 15, 16, 19, and 21
8	1989	5	Vascularized calvarial bone flap	No additional procedures
9	1990	5	Vascularized calvarial bone flap	No additional procedures
10	1992	5	Vascularized calvarial bone flap	Required bone grafting at age 15 and 17

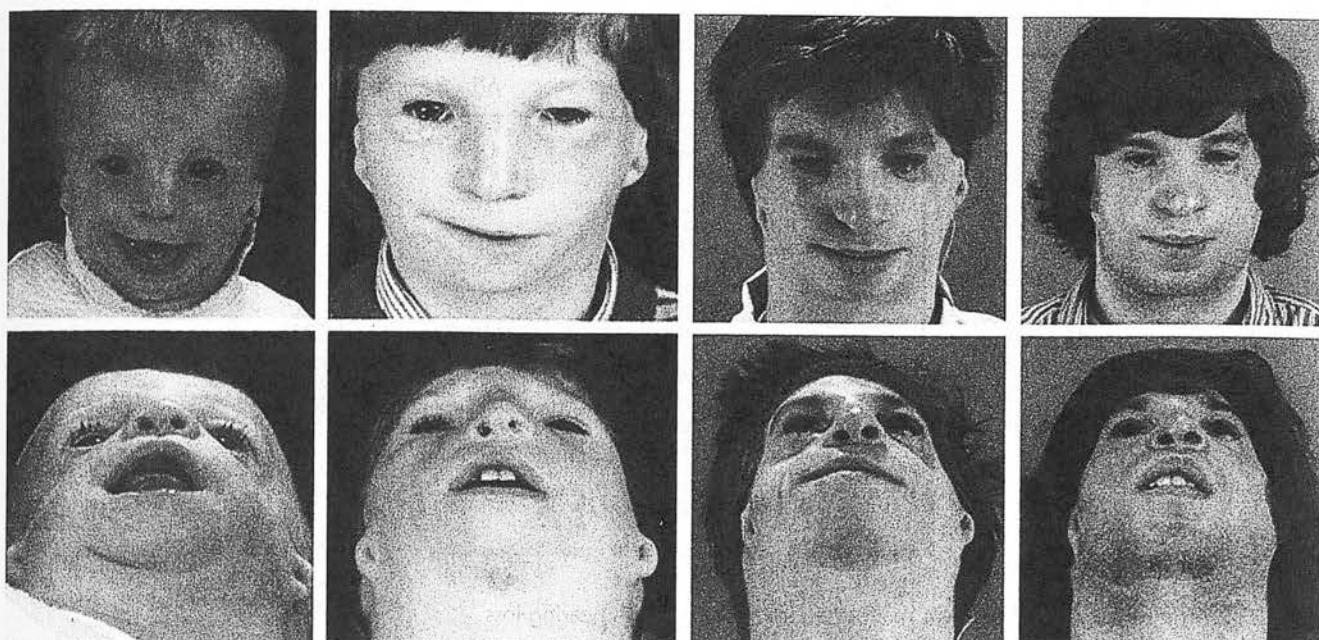


FIGURE 3. Birth to maturity: Note the improved facial contour after the initial reconstruction using a vascularized temporalis bone flap followed by recurrence of the deformity and autologous bone grafting in adolescence.

Collins syndrome have a myriad of complex problems that are all interrelated, these patients are best treated through a centralized unit where all of the various disciplines can coordinate their activities based on the overall picture of a birth to maturity protocol. This review demonstrates the complexity of care that patients with Treacher Collins syndrome need and has led to the development of a protocol to help guide care in the future (Fig. 4).

The protocol for management used at the Australian Craniofacial Unit can be summarized in 3 epochs. During the first epoch from birth to age 2, the focus is on vital issues such as airway management and nutrition. Feeding difficulty and failure to gain weight are often the primary symptoms of airway compromise.⁷⁻⁹ A variety of interventions can be implemented including prone or side positioning, nasopharyngeal airway, and nasogastric feeding.

TABLE 4. Procedures and Details in 10 Patients Who Underwent Orthognathic Surgery

Patient No.	Age, y	Occlusion	Maxillary Procedure	Mandibular Procedure
1	16	Class II div I, anterior open bite	None	Sagittal split osteotomies; 7-mm advancement
2	13	Class II div I, anterior open bite	Le Fort I; 5-mm posterior impaction	Sagittal split osteotomies; 4-mm advancement
3	17	Class III, anterior open bite	Le Fort I; 4-mm advancement	Sagittal split osteotomies; 5-mm setback
4	18	Class III, anterior open bite	Le Fort I; 10-mm advancement, posterior impaction	None
5	19	Class I, anterior open bite	Le Fort I; 6-mm advancement/posterior impaction	C osteotomies; 6-mm advancement
6	15	Class II div I, anterior open bite	Le Fort I; 5-mm advancement	Sagittal split osteotomies; 8-mm advancement
7	22	Class II div I, anterior open bite	Le Fort I; 5-mm advancement, posterior impaction	C osteotomies; 10-mm advancement
8	29	Class II div I, anterior open bite	Le Fort I; 3-mm setback, posterior impaction	C osteotomies; 5-mm advancement
9	19	Class II div I, anterior open bite	Le Fort I; 5-mm advancement, 4-mm downward rotation	C osteotomies; 8-mm advancement
10	19	Class II div I, anterior open bite	Le Fort I; posterior impaction	Sagittal split osteotomies; 7-mm advancement

Treacher Collins Protocol – Australian Craniofacial Unit		
First Epoch	Newborn Respiratory/Neonatal physician Craniofacial surgeon ENT Social work Speech pathologist	Respiratory management Airway management, consideration of distraction Airway management, consideration of tracheostomy Introductory appointment, support for parents Initial appointment as soon after birth as possible to monitor feeding progress
	First three months Craniofacial surgeon Speech pathologist Ophthalmologist ENT and Audiology Craniofacial registrar Dentist Photography Genetics	Consultation regarding protocol management Review feeding progress, introduction to speech management Evaluate vision, corneal exam Check ears and arrange for hearing aid devices as needed Take relevant medical history details Discuss dental development and care Initial photos Initial genetic counselling
	Three months to second year ENT Audiology Ophthalmologist Orthodontist Speech pathologist Photography Craniofacial surgeon	Assessment 6 mo following surgery if grommets inserted; review at age 1 yr Track hearing status, manage hearing aids Yearly Review Consultation to outline management protocol Review at 1 year to check speech and language development Yearly Review; palate surgery if indicated
Second Epoch	Second through fifth year Respiratory Ear, nose, throat surgeon Audiology Dental Speech pathology Radiographs Orthodontics Ophthalmologist Social worker / psychologist Photography Craniofacial Surgeon Craniofacial Clinic	Evaluation / sleep study as needed Review yearly Review as indicated Review yearly Assessment at 18 mo, 2.5 yr, 3.5 yr, 4.5 yr Cephalometry at age 4.5 yr to plot facial growth Introduction Review yearly Assessment and intervention as indicated Yearly, pre and post op Repair of zygomatic/orbital clefts and eyelids Attendance at age 18 mo and age 4 yr
	Sixth through twelfth year Respiratory Ophthalmology Dental Orthodontics Ear, nose, throat surgeon Audiology Speech pathology Nasoendoscopy Radiographs/ Photography Social work Craniofacial surgeon Craniofacial Clinic	Evaluation as needed Review yearly Review yearly Assessment yearly Review yearly Review as indicated Assessment at age 8yr and 12 yr If recommended Cephalometry yearly from age 6 yr Assessment and intervention as needed Review yearly; otoplasty, touch-up surgery Attendance at age 8 yr and 12 yr
Third Epoch	Thirteenth through eighteenth year Respiratory Ophthalmology Dental Orthodontics Ear, nose, throat surgeon Audiology Speech pathology Social work Radiographs Photography Craniofacial surgeon Craniofacial Clinic	Evaluation as needed Review yearly Review yearly Assessment yearly Review yearly Review as indicated Review as indicated Review yearly Cephalometry yearly Yearly Review yearly; Orthognathic surgery, Revision/ repeat bone grafting to zygomatic clefts Attendance 18yr for final assessment

FIGURE 4. The Treacher Collins protocol at the Australian Craniofacial Unit.

Results here indicate that most airway problems can be managed with positioning or with a nasal airway. Once a tracheostomy has been placed, however, it was difficult to remove and required childhood distraction in all cases. Neonatal distraction was not used

here but could be considered in severe cases to avoid tracheostomy. In this series, 10 patients had breathing difficulties into childhood including 2 who had no problems in infancy. This illustrates the need for continued observation throughout development. Finally, the

difficult airway in patients with Treacher Collins syndrome should not be underestimated. Experienced anesthetists play a critical role in the multidisciplinary care.

Hearing loss is another important issue that is addressed during the first epoch. Because 17% of patients with normal-appearing external ears still had at least a mild hearing impairment, external ear appearance should not influence audiometry screening. In fact, the more normal appearing the external ear, the more likely the child will benefit from an intervention such as tympanostomy tube placement. Middle ear reconstruction can be considered for patients with Treacher Collins syndrome if deemed feasible when the child is older. However, the goal of ossicular reconstruction should be to achieve hearing without the need for aids, which was not accomplished in 1 patient who had this done in this series. Any otologic surgery planned should be performed by a surgeon familiar to the abnormal anatomy of the facial nerve in Treacher Collins syndrome.¹⁰

Genetic counseling is offered to patients and families during the first epoch. Genetic testing is possible and has identified mutations from 48% to 93% of patients thought to have Treacher Collins depending on the how extensive the genetic screening and the clinical accuracy of the patient population tested.^{11–14} In this study, 2 of 4 patients tested positive for the *TCOF1* gene. Because this mutation is not present in similar conditions such as Goldenhar, Miller, or Nagar syndrome, the role of testing may be best suited to patients who have an equivocal clinical diagnosis.^{13,15,16} Based on the cost of testing, and the relative ease of clinical diagnosis, genetic testing is not a routine part of the Treacher Collins protocol.

The second epoch, from ages 2 to 12 years, focuses on speech, social development, and the primary surgical reconstruction of the upper face. Facial reconstruction procedures for Treacher Collins syndrome have been described in detail before.^{9,17–20} In this series, a combination of autologous bone grafting and vascularized bone flaps was used. Neither technique produced lasting results, leading to repeated bone grafts in two-thirds of the patients. Most patients treated after 2000 underwent reconstruction using autologous bone grafts, and the protocol reflects the need for repeated grafting later in life. The lower eyelid deformity was also treated at this time using a musculocutaneous transposition flap from the upper eyelid to the lower combined with a lateral canthopexy. Although generally achieving the desired effect of protecting the cornea from exposure, the cosmetic results remain one of the most difficult aspects of managing Treacher Collins.

Previously, it has been shown that the teasing craniofacial patients receive is not necessarily specific to their deformity or its severity.²¹ Nevertheless, when faced with criticism or teasing, most children have coped remarkably well.²² Therefore, the timing of surgical intervention in this protocol is not driven by teasing. Teasing can usually be managed through education of the parents, teachers, and peers of the patients rather than deviation from the treatment plan. This helps them to understand what is happening at each point in development and eliminates unnecessary surgical interventions.

Speech and language development must be watched closely during this period. Generally, resonance and articulation problems improved with therapy, structural growth, and orthodontic correction. However, it is important to monitor articulatory proficiency from an early age to detect maladaptive compensatory patterns and intervene where appropriate.

In the third epoch, from age 12 to 18, the facial reconstruction is finished. During this time, orthognathic surgery, rhinoplasty, and genioplasty operations were performed. Ideally, these operations should be combined when possible, although when a Le Fort I osteotomy was performed, the rhinoplasty was performed separately. Because repeat zygomatic bone grafting is often required, it should

be performed in conjunction with other procedures. In this experience, all of the patients undergoing orthognathic surgery required correction of an anterior open bite along with variable amounts of horizontal malocclusion. The anterior open bite was corrected in all cases, and no patients required repeat surgery for this problem.

Formulating outcome goals for patients with such a wide variety of problems can be difficult. Nonetheless, it is critical to the overall management strategy to have a vision of what can and should be achieved throughout growth and development. Clearly, within each subspecialty, the outcomes will be dependent on the severity of the deformity. The goal of therapy, however, is the same. Each patient should have an opportunity to overcome as much of the deformity as possible. In the future we plan to generate a survey of the protocols and outcome goals of other units around the globe as we move forward in the benchmarking process through the International Society of Craniofacial Surgery.

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Longitudinal outcome of pharyngoplasty

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Keywords

Cleft palate, complications and modifications, pharyngoplasty.

Abstract

Although early complication of airway obstruction following pharyngoplasty is well recognised, there have been few reports of late modifications following this procedure. We retrospectively review cases with late complications which have required either revision or division of an existing pharyngoplasty at the Australian Craniofacial Unit over the last twenty-five years. We assess the outcome of further surgical intervention in each case, with case note and nasendoscopy video review. Fourteen cases were identified where records were complete. There were 12 males and 2 females. The cases are a heterogeneous group of cleft lip and palate patients and include three cases with a diagnosis of Pierre-Robin sequence and one case with a cleft palate as part of an underlying syndrome. Those cases requiring flap division had undergone either superiorly or inferiorly based pharyngeal flaps in contrast to dynamic (Orticochea) pharyngoplasties which required revision. This series of cases demonstrates the need for thorough assessment and planned tailoring of the pharyngoplasty procedure, with ongoing review of speech and airway function. This management philosophy results in the acceptance that a pharyngoplasty may only be required for a limited period of time and ultimately may be redundant.

Introduction

The development and application of high-resolution endoscopy to the study of velopharyngeal function has revolutionized the management of velopharyngeal dysfunction (David & Bagnall, 1990). Prior to its availability standard surgical treatment of velopharyngeal incompetence (VPI) was confined to either flap or sphincter pharyngoplasty. Generally the chosen technique was based on fluoroscopic analysis of velopharyngeal activity and the surgeon's clinical judgement and experience. It was then standard practice to leave the pharyngoplasty untouched except in the case of airway obstruction.

With improved imaging and visualization it has become possible to analyse all components of the velopharyngeal mechanism and tailor surgery to suit the dysfunction (David *et al.* 1982). Longitudinal experience by clinicians dedicated to achieving excellence has led to an understanding of the need for continued refinement. This is particularly so when initial velopharyngeal surgery is

undertaken during childhood before the completion of growth and maturation of the motor speech system. We present fourteen cleft palate cases that had undergone pharyngoplasty and who have subsequently required either modification or had it taken down. We discuss their management, including the indications for further surgical intervention, and assess its outcome.

Materials and methods

Cases of cleft palate who had undergone revision or division of a pharyngoplasty were identified from the Australian Craniofacial Unit database. Case note review (along with video records of the nasendoscopy studies), were undertaken in all cases.

Results

Fourteen cases were identified where records were complete. Details of the cases presented are summarized in Table 1. There were 12 males and 2 females. Seven cases had isolated cleft palate, 3 had submucous cleft palate, 2 had unilateral cleft lip and palate and 2 had bilateral cleft lip and palate. In addition, three cases had Pierre Robin sequence and 1 case had Stickler syndrome. Four cases had primary pharyngoplasty surgery at other centres. Those who had undergone Orticochea pharyngoplasty

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Table 1: Summary of the cases

Case no.	Sex	Diagnosis	Pharyngoplasty type	Age (Years)	Revision/ Division	Age (Years)	Time to Revision (Years)
1	m	ICP	Sup. Pharyngeal flap	4*	D	10	6
2	f	ICP-Stickler	Sup. Pharyngeal flap	8	D	18	10
3	m	ICP-PR	Sup. Pharyngeal flap	5	D	14	9
4	m	SMCP	Sup. Pharyngeal flap	14	D	19	5
5	m	UCLP	Sup. Pharyngeal flap	10	D	31	21
6	m	ICP	Sup. Pharyngeal flap	5*	D	11	6
7	m	ICP	Sup. Pharyngeal flap	10	R	16	6
8	m	SMCP	Orticochea	4	R	16	12
9	m	BCLP	Orticochea	7	R	18	11
10	m	ICP-PR	Orticochea	4	R	7,10,14	3, 3, 4
11	m	ICP-PR	Orticochea	10	R	17	7
12	f	SMCP	Orticochea	5	R	9	4
13	m	BCLP	Inf. Pharyngeal flap	17*	D	39	22
14	m	UCLP	Inf. Pharyngeal flap	7*	D	17	10

*Pharyngoplasty performed elsewhere

Diagnosis: ICP = Isolated Cleft Palate
 SMCP = Submucous Cleft Palate
 UCLP = Unilateral Cleft Lip and Palate
 BCLP = Bilateral Cleft Lip and Palate
 PR = Pierre Robin sequence

(Orticochea, 1968) required revision, while those with superiorly or inferiorly based pharyngeal flaps underwent division in all cases except one.

Discussion

There has been few reported cases about the need for revision following pharyngoplasty. We have identified a group of cases all of which initially benefited from their pharyngoplasty, with no case requiring any intervention in less than three years following primary surgery. This is different from reports from other centres where the initial surgery has failed (Ma *et al.* 1996) or required revision within two years of the primary surgery (Kasten *et al.* 1997, Pryor *et al.* 2006).

The cases presented in this series have had different indications for modification of their pharyngoplasty. The cases are heterogeneous cleft palate population and the only finding of note was that most of isolated cleft palates had the Pierre Robin sequence. However, this heterogeneous population can largely be divided into three groups as to the reason for further interventions: airway obstruction alone, speech difficulties (unresolved VPI), and combined airway obstruction with speech difficulties. Each case will be considered according to the indication for revision.

Group 1. The smallest group consisted of just one subject (case 5) who required modification to the pharyngoplasty due to a compromised airway. This subject had a repaired UCLP and at age 10. He underwent a superior pharyngeal flap following assessment of nasal air escape affecting speech and confirmed by nasendoscopy. He re-presented at age 30 with recent history of sleep apnoea requiring C-PAP. Speech was assessed as mildly to moderately de-nasal with no evidence of VPI or hypernasality. Nasendoscopy demonstrated severe nasopharyngeal

obstruction (Figure 1). The components of the velopharyngeal sphincter were noted to be functioning appropriately during speech and the flap was deemed redundant. He subsequently underwent division of the flap and on reassessment he no longer complained of snoring, did not fall asleep at work and the sleep study was normal.

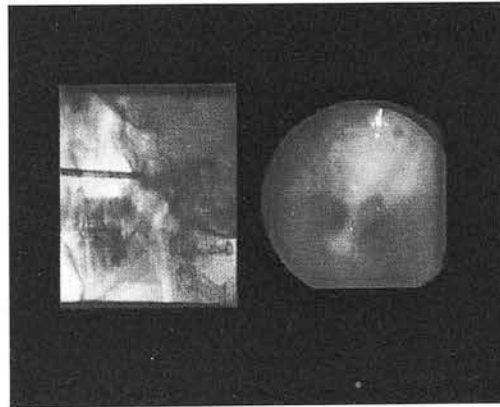


Figure 1: Case 5 Nasendoscopy age 31 demonstrating low attachment of the superior pharyngeal flap at rest prior to division.

Group 2. The largest group which had a total of eight subjects who required surgical revision as part of their management of VPI (cases 4,7,9,10,11,12,13,14).

Case 4 in this group had a superiorly based pharyngeal flap at age 14 after submucous cleft palate repair. This young male had a mild intellectual deficit and considerable debate took place prior to recommending surgery as nasendoscopy demonstrated minimal muscular effort. His poor speech had contributed to his social isolation and severe expressive language difficulties. Eventually it

was agreed that the purpose of the flap would be to partially obturate the isthmus, thus increasing vocal energy and loudness, providing this young man with more intelligible conversational speech. The desired outcome was achieved but the flap was divided four years later at the time of Le fort 3 maxillary advancement. Post-operative assessment including nasendoscopy did not demonstrate any deterioration, which have been previously noted (Harries *et al.* 1992).

Case 7 had a superiorly based pharyngoplasty following assessment of gross hypernasality and audible nasal air emission supported by observations at nasendoscopy. Post-operative hypernasality was improved and assessed as mild. Six years later after speech deteriorated, the flap was revised. Post-operative assessment demonstrated mild denasality, no hypernasality but minimal nasal air emission through a palatal fistula. The patient declined further surgery.

Three cases (9, 10 and 11) had Orticochea sphincter pharyngoplasties performed at ages 4, 7 and 10 years respectively, with nasal air emission accompanying speech and assessed as ranging from mild to moderate. The surgery resulted in reduced nasal air emission but none was fully competent. Case 9 underwent a superiorly based pharyngeal flap eleven years after the Orticochea in an effort to fine-tune the closure. Case 10 had undergone two posterior pharyngeal wall implants, of which the second time was with pre-operative tattooing (Maegawa *et al.* 1998), coordinated with therapy for hypernasality. His speech improved and assessed as normal in resonance with effective sphincter closure. Speech assessment four years later demonstrated VPI and recurrence of hypernasality, which appeared to coincide with a period of teenage skeletal and soft tissue growth. Nasendoscopy demonstrated incompetence and he underwent a revision of the Orticochea pharyngoplasty and a short follow-up course of resonance therapy. Review one year later confirmed no nasal air emission or hypernasality. Case 11 underwent nasendoscopy seven years after his Orticochea pharyngoplasty because his speech was noted to be less intelligible. Endoscopy revealed the left flap had become detached. This was surgically re-positioned and his speech clarity improved.

Case 12 was referred for investigation of a speech disorder and subsequently diagnosed with a submucous cleft palate. She underwent Veau-Wardill-Kilner cleft palate repair. Post-operatively nasendoscopy demonstrated a central defect but good lateral pharyngeal wall movement. On-going speech reviews reported limited speech improvement so one year later the patient underwent an intravelar veloplasty and Orticochea pharyngoplasty. The following year increasing velopharyngeal incompetence and hypernasality was noted. A nasendoscopy

found the pharyngoplasty was functioning well but the tonsils were large and inhibiting velar elevation. A tonsillectomy was performed. Post-operatively, although improved, speech featured nasal air emission on the production of high pressure oral phonemes. Two years later nasendoscopy demonstrated maintained function but the velopharyngeal gap was significant and central pharyngoplasty was recommended. This was undertaken 2 weeks later and speech review four months post-operatively found tight velopharyngeal closure on demanding testing, marked improvement in intelligibility but persisting mild assimilative hypernasality. She remains under review.

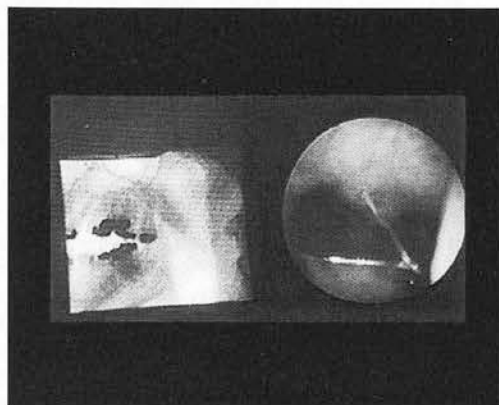


Figure 2: Case 13 Nasendoscopy age 39 years demonstrating the inferior pharyngeal flap at rest with its low attachment prior to division.

Two cases (13 and 14) had inferiorly based central flaps. Both had primary early cleft management and pharyngoplasties in other centres (they first presented at 17 and 39 years of age respectively). Tethering of the velum was observed at nasendoscopy in both cases (Figure 2), and both underwent division of the flap. Post-operative nasendoscopies clearly demonstrated improvement in velar activity with firm or touch closure. Speech assessment supported the finding of nasendoscopy and recommended therapy to decontaminate the speech of hypernasality. While there are a relatively large number of superiorly based pharyngeal flaps in this series (seven), this procedure was commonly undertaken by the department during the period of study. In contrast the two cases of inferior pharyngeal flaps in this series were the only examples managed in the unit during the period of study. That both of these cases required division suggests that this flap could be particularly prone to requiring later modification.

Group 3. The five cases in this final group underwent surgery for combined airway and speech difficulties (cases 1, 2, 3, 6 and 8). Case 1 had a superior pharyngeal flap at age 4 years at another centre and nasal air emission and hypernasality were reportedly eradicated.

Speech assessment at this Unit at age 10 years revealed moderate to severe denasality and snoring. Nasendoscopy demonstrated a wide flap obstructing the nasopharynx and tethering the velum. All components of the mechanism worked well suggesting competence would probably be maintained if the flap was divided. Speech assessment following division revealed no hypernasality, mild denasality and no detectable nasal air emission. Snoring was eradicated.

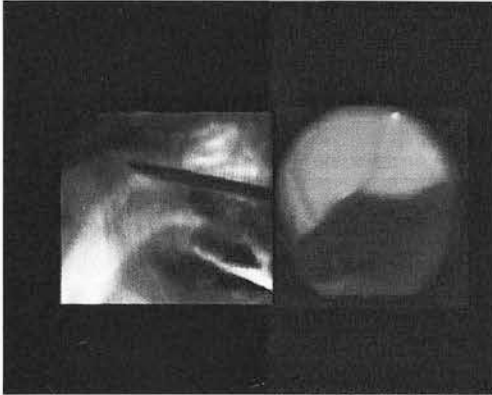


Figure 3: Case 2 Nasendoscopy at age 7 years demonstrating large gap during speech.

Case 2 had a superiorly based pharyngeal flap at age 8 following identification of a velopharyngeal defect during nasendoscopy (Figure 3). Post-operative nasendoscopy was satisfactory. The patient presented at age 18 for investigation of VPI and snoring. Nasendoscopy demonstrated a wide flap with small lateral ports compromising breathing (Figure 4), and speech assessment identified a mixed resonance disorder with denasality and audible nasal air emission accompanying sibilants. The pharyngoplasty was divided and post-operative speech assessment confirmed improvement with a reduction in de-nasal resonance and snoring, and an increased nasal airway for breathing.

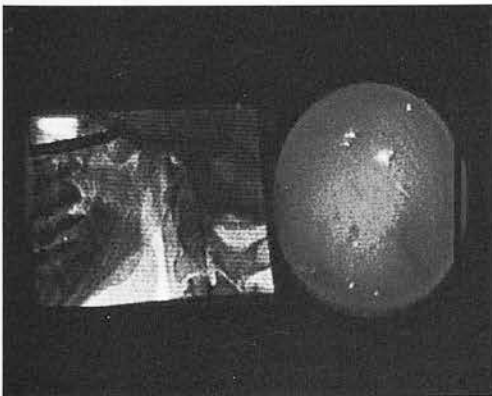


Figure 4: Case 2 Nasendoscopy following pharyngoplasty at age 17 years demonstrating diminutive lateral ports at rest.

Case 3 demonstrates issues inherent in a central flap pharyngoplasty in cases with a diagnosis of Pierre-Robin syndrome. This boy was treated at another center and had his primary palate repaired at age 16 months and a pharyngoplasty due to severe velopharyngeal incompetence at age 5 years on the speech pathologist's recommendation. The surgeon chose to provide a superior pharyngeal flap. Over the next few years his speech improved with eradication of nasal air emission but denasality gradually began to dominate his speech pattern. A speech assessment nine years later in this unit when the patient was aged 14 years identified severe denasality and reports of consistent severe snoring. Nasendoscopy demonstrated small ports and obstruction due to the flap. Five weeks later the flap was divided and post-operative speech assessment at 3 months noted reports of clearer speech, a reduction in snoring, mild denasality and velopharyngeal competence.

Case 8 first presented at age 4 years with an unrepaired submucous cleft palate. He had simultaneous palate repair and Orticochea pharyngoplasty. Six years later he gave a history of increasing denasality and underwent a nasendoscopy. Revision was recommended but not carried out. He presented twice more during childhood and received similar advice. At age 16 he finally underwent pharyngoplasty revision during which the Orticochea flaps were re-positioned superiorly. Post-operative nasendoscopy and speech assessment confirmed improved airway with adequate competence for speech and perceptible improvement to speech intelligibility. He remains under review.

The last case in this group (Case 6) was again initially treated elsewhere. His soft palate cleft was repaired at 6 months of age and the un-repaired hard palate defect was obturated, though not successfully, for speech. At age 5 years he underwent hard palate repair and simultaneous central pharyngoplasty. Reported speech improvement but persisting velopharyngeal incompetence was noted. He attended this Unit at age 10 years for an initial assessment that revealed de-nasal speech, hypernasality and nasal air emission. Nasendoscopy findings confirmed that the superior pharyngeal flap was attached too low to be effective. Given that the patient had good lateral pharyngeal wall movement it was decided to divide the flap at the same time as the palatal fistula was repaired. Speech review ten months later reported no change in hypernasality or nasal air emission but eradication of denasality and reliance on mouth breathing. He is due to undergo nasendoscopy to plan the next phase of management.

Conclusion

A series of heterogeneous group of cleft palate patients who have undergone pharyngoplasty

and a subsequently required surgical modification were reviewed. Results showed that the surgery undertaken, superior (and especially) the inferior, pharyngeal flaps may become redundant and require taking down. However, the Orticochea flaps may require revision of the position of their flaps. Following these surgical interventions the speech has been improved but has not achieved complete resolution of symptoms in all cases.

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CHAPTER THREE

INTRODUCTION

Trauma to the facial skeleton in children has been regarded as relatively uncommon in comparison to adults. There are differences in the shape and bone quality of the Craniofacial skeleton as well as differences in behaviour and activities to account for this. The differences in anatomy due to relative immaturity of the lower and mid face, the development of the dentition and the sinuses all impact on the fracture patterns and have a risk of long term growth disturbances affecting the craniofacial skeleton. Contemporary behavioural changes in western societies has included copying the violent behaviours of their elder peers. This has had a significant contribution on the incidence of injuries in young teenagers, where there has been a six fold increase in paediatric facial trauma at Adelaide Children' s hospital during the period 2005 to 2010.

This series of papers looks at the aetiology, incidence and patterns of facial fractures in children. They also consider the implications for management as a result of the different facial anatomy in children, and highlight the potential for disturbances in facial growth which although is well established following fractures of the mandibular condyle¹, is poorly recognised following fractures in other parts of the facial

skeleton, and may not become apparent for several years after the initial injury.

1. Treatment of Fractures of the mandibular condylar processes in Children (1965)

MacLennan WD, Simpson W. British Journal of Plastic Surgery **18**:
423 - 427.

CHAPTER THREE - PAPERS

The first paper is a ten year review of paediatric fractures presenting to a children's regional trauma centre. It had the advantage in that all facial injuries were treated at this centre, but they were managed by six different surgical disciplines. All departments agreed to participate in a unified study enabling an examination of the incidence, aetiology and fracture patterns sustained in a ten year period, producing a comprehensive study. This found nasal fractures the most common injury, which are often excluded in other reviews. Overall, there was a much higher than expected incidence of associated injuries. The detailed study was selected for citation in the 1995 year book of Otolaryngology, and has since been cited over 50 times by later authors studying pediatric facial injuries.

The second paper is two case reports of orbital floor fractures in young children and demonstrates the pattern of orbital floor injuries in children where the maxillary sinus development is immature and the resulting orbital floor bone much thicker. The history, clinical signs and surgical management of these unusual cases are reviewed.

The third paper is a demonstrates that localised growth anomalies can occur in the mandible following a fracture at a site other than the condyle. In these cases parasymphiseal fractures in children where the fracture involves the developing canine tooth which is close to the

lower border of the mandible. These subsequently develop localised overgrowth which disturbs chin point position and can be treated by a genioplasty at skeletal maturity.

The fourth paper is a report of an unusual aetiology for an injury in an infant where the bones of the facial skeleton are thin and fragile. In this case facial fractures were produced as a consequence of a dogbite which would not be expected to produce such injuries in an adult. It highlights the need for high index of suspicion of injury and careful evaluation in children.

The fifth paper is a demonstration of the difficulties in conventional radiological examinations in attempting to establish the full extent of injuries in a child. The plain radiology failed to identify a number of fractures and failed to demonstrate the full extent of other fractures. This case used high resolution CT scanning to aid diagnosis and so enabled comprehensive treatment. It highlights that CT assessment of injured children with complex injuries may be necessary for accurate diagnosis.

The sixth and final paper is a review of an injury which had received little attention prior to its publication. Medial blow out injuries have only become well recognised in adults with the advent of CT scanning. In children there had been only isolated reports but this report of the clustering of seven cases in two years in one centre suggests the true incidence is much higher than the current published literature suggests.

The histories, clinical findings and management of are reviewed and act as a guide for clinicians to suspect this fracture pattern in other injured children.

In summary these papers widen the knowledge of the presentation and aetiology of apparently uncommon injuries, and demonstrate that growth disturbances can occur after fractures other than the mandibular condyle. However, further prospective studies are required to evaluate the risks of such fractures resulting in a growth disturbance during development.

Fractures of the facial skeleton in children

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Fractures of the facial skeleton in children are uncommon. This study presents the results of 139 children who sustained a total of 161 such fractures and were admitted to the Royal Hospital for Sick Children, Edinburgh, between January 1983 and December 1992.

The male to female ratio was 3:1 and the highest incidence was at age 10 years. Analysis of fracture patterns showed that despite differences in anatomy, the fracture patterns were similar to those occurring in adults, but the relative proportion of each fracture type was different in children. Nasal fractures occurred most frequently (54 per cent), mandibular fractures constituted 30 per cent, and middle third fractures only 16 per cent.

Falls, sporting injuries and road traffic accidents (RTA) were the major causes of these injuries. Injuries sustained in RTA were most likely to have involved cyclists or pedestrians in contrast to earlier series which have identified these injuries mainly among car passengers.

No deaths were recorded and most patients made a complete recovery, although a few required secondary surgery for complications. This taken in conjunction with the findings of both high numbers of associated injuries, and increased severity commonly occurring in both mandibular and middle third injuries leads to the suggestion that these should be treated in centres where multidisciplinary management can easily be coordinated.

The aim of this study was to collect data pertaining to all children with fractures of the facial skeleton from a UK Centre, to look specifically at the nature, incidence and aetiology of these injuries and compare the results with published data from other countries.

Methods

This retrospective study acquired data from this hospital's records department for the 10 year period from 1 January 1983 until 31 December 1992. Patients who had a discharge coding of facial fracture had their case notes reviewed and, if a facial fracture was documented on physical findings or reported on radiographic investigation, were included in the study. Where the findings were unclear the radiographs were reviewed and doubtful cases eliminated. One set of case notes had been destroyed and this too was excluded from the study.

At this children's hospital, patients were normally seen until their thirteenth birthday, after this age they would be treated as adults in one of several centres. This centre had the advantage in that all children from South-East Scotland with facial injuries requiring inpatient care were treated here.

Results

One hundred and sixty-one fractures of the facial skeleton occurred in 139 patients during the period of study. Two patients were admitted twice during the period of study with different injuries and were counted as separate events. There was little change in the incidence when different years within the study were examined and no detectable seasonal difference within each year. The age range was from 6 months to 13 years and 3 months. The exact incidence in relation to age can be seen in Table I, but it can be seen that the peak incidence was at age 10 years with

Table I. Ages of children with facial fractures (N=139)

Age	N	Age	N
<1	2	7-8	15
1-2	2	8-9	15
2-3	8	9-10	14
3-4	8	10-11	19
4-5	4	11-12	15
5-6	9	12-13	15
6-7	10	13+	3

Injury, 1995, Vol. 26, 47-50, January

Introduction

Fractures of the facial skeleton are uncommon in childhood, in comparison both with other bones of the body and with the incidence in adults. This is thought to be due in part to the relatively smaller size of the face and the elasticity of the bones¹. With the development of the dentition within the smaller jaws of a child, and of the paranasal sinuses during childhood, it might be expected that the patterns of fracture would be different to those occurring in the adult skeleton.

Within the UK the treatment of these injuries is within the remit of several surgical specialties and which specialty is involved in management may depend on local circumstances. Within this hospital the following surgical specialties were involved in the management of facial fractures: paediatric surgery, plastic surgery, maxillofacial surgery, ENT surgery, ophthalmology and neurosurgery. This fragmentation of care has been repeated at other centres and has resulted in an absence of published UK data on the overall incidence and aetiology of these injuries, although UK data relating to children has been included in some series of mandibular and middle third fractures²⁻⁵.

relatively fewer cases (only 17 per cent of the total) occurring in those aged 5 years and below. There was a marked difference between the sexes with 74 per cent occurring in males. This difference was maintained in all fracture types.

The types of injury sustained were divided into the following categories and the frequency of each type is shown in brackets: nasal fractures (54 per cent), mandibular fractures (30 per cent) and middle third (excluding nasal) fractures (16 per cent). Five patients had multiple fractures occurring in two categories, they have been counted only once as patients for overall numbers but each fracture has been included in the relevant category when examining fracture types.

Nasal fractures were noted in 87 patients admitted to the hospital some of whom were admitted primarily for the treatment of other injuries. Mandibular fractures occurred in 34 patients, producing a total of 48 fractures. The types of fracture seen were similar to those occurring in adults. The sites of fracture are shown in Table II. Middle third fractures included those patterns occurring in adults. No patient had more than one middle third fracture. Surprisingly, the maxillary fractures were all of the Le Fort 2 type but the numbers were small. However, all the middle third fracture types are found in the adult skeleton but there was higher proportion of isolated orbital fractures present than might have been expected in an adult population. The full results showing the incidence of different types of middle third fractures can be seen in Table III. It is also noteworthy that only five of the total of 13 dentoalveolar fractures in this series occurred in the maxilla.

Associated injuries were recorded from the notes and most commonly were head injuries and facial lacerations. However, while limb fractures (including humerus, radius, ulnar, metacarpal and phalanges in the upper limb, femur, tibia, fibula and calcaneum in the lower limb) and abdominal injuries (including ruptured spleen and liver laceration both requiring laparotomy) were noted, no chest injuries or cervical spine injuries were observed. When comparing the frequency and severity of associated injuries with the facial fracture type, it was shown that these were most likely to be associated with mandibular fractures and to a lesser extent middle third fractures, as shown in Table IV.

The aetiology of each injury was recorded from the patients' case notes and the different causes are shown in Table V. Falls were the most common cause, closely followed by sporting injuries and road traffic accidents. Closer study of the sports involved was undertaken to establish common causes. While golf and horses were the most frequent causes a large number of sports (total 13) were involved. The results are shown in Table VI. Detailed study of the type of the road traffic accident resulting in

injury are shown in Table VII. This revealed that these were largely occurring to cyclists or pedestrians, with few injuries occurring to passengers in vehicles. It was noted that neither of the patients who were riding motor cycles were wearing safety helmets.

Treatment involving operative surgical intervention was performed in 92 per cent of nasal fractures, 82 per cent middle third fractures and only 32 per cent of mandibular fractures; the rest were treated non-operatively.

Table II. Types of mandibular fracture

<i>Sites of fracture</i>	
Condyle	20
Body	11
Parasymphysis	5
Symphysis	4
Dentoalveolar	8

Total $N=48$; unilateral $N=20$; 2 fractures $N=11$; 3 fractures $N=2$.

Table III. Types of middle third fractures (total $N=26$)

Le Fort 2	3
Malar	8
Isolated orbital rim	6
Dentoalveolar	5
Nasoethmoid complex	2
Isolated antral wall	1
'Blow out' orbital fracture	1

Table V. Causes of facial fractures ($N=139$)

	<i>N</i>	<i>%</i>
Fall	41	30
Sports	38	27
Traffic accidents	32	23
Assault	14	10
Playground	14	10

Table VI. Sports resulting in injury ($N=38$)

Golf	8
Horses	6
Soccer	6
Gymnastics	4
Swimming	3
Others (8 in total)	11

Table IV. Associated injuries

	<i>Other injury</i>	<i>Head injury</i>	<i>Lac.</i>	<i>Limb fracture</i>	<i>Abdominal trauma</i>
Mandibular fracture $N=33$	21 (65%)	9	15	6	2
Middle 1/3 fracture $N=24$	12 (50%)	8*	7	—	—
Nasal fracture $N=87$	19 (23%)	6	9	4	—

Lac. = laceration.

*Three cases had associated mandibular fractures.

Table VII. Traffic accidents resulting in injury ($N = 32$)

Cyclists	14
Pedestrian	13
Vehicle passengers	3
Motorbike	2

The timing of surgical intervention for nasal fractures was found to range from almost immediately following admission to 16 days after injury. The peak time for nasal manipulation was within 2 days of admission, although there was a second peak at 7 days after injury; overall 72 per cent were operated upon within 7 days of admission. One patient required re-operation and three patients were noted to have a less than ideal result which may require rhinoplasty once adulthood is reached.

The treatment of the mandibular fractures was related to displacement and stability. Those fractures occurring at the body, symphysis or parasymphysis, and which were displaced, proceeded to operative reduction. In the early part of the study this was by closed reduction using a MacLennan splint which fits over the teeth and is held in place by perimandibular wiring (total seven). Later on, these fractures were treated by open reduction with position maintained by Luhr miniplates (total four). Those occurring at the neck of the condyle were treated non-operatively by soft diet, the occlusion being satisfactorily maintained in all cases. Those at other sites which were undisplaced and stable were treated in a similar manner. The dentoalveolar fractures were treated by reduction and splintage using a polypropylene dental splint to maintain position.

Middle third injuries were largely treated with the same method as in adults. The maxillary fractures were treated with arch bars, circum-zygomatic wiring and intramaxillary fixation. The depressed malar fractures were elevated via Gillies' temporal approach but the procedure was modified in that a Howarth's periosteal elevator substituted for a Bristow's elevator. The nasoethmoidal complex injuries were treated by open reduction, while the 'blow out' fracture had early exploration of the orbital floor and silastic implant. The isolated orbital rim injuries and the antral wall fracture were treated non-operatively. The dentoalveolar fractures were treated in the same way as those occurring in the mandible, and were described in the previous paragraph.

While no deaths are recorded, a variety of clinically significant long-term complications following injury were noted. These included persistent leakage of cerebrospinal fluid requiring two craniotomies 1 year after injury, and persistent diplopia 4 years following injury and thought unlikely to improve. Facial scarring was sufficiently severe in one case to be still under treatment 8 years following injury (and which had already been the subject of four operative interventions). Infected facial wiring required removal 6 years after surgery. Persistent periorbital swelling due to a blocked tear duct required dacryorhinocystostomy 2 years after injury. No long-term disturbances in facial growth are recorded in this series but few patients have had long-term follow up.

Discussion

This study confirms findings from earlier studies that facial fractures in children are uncommon and that there has been

no change in their incidence in the period of the study. No seasonal differences in incidence were noted and this contrasts with a previous investigation of mandibular fractures which found these to occur most commonly in summer⁶. However, there is little comparative data currently available and the importance of this is unclear but may reflect the different mechanism of injury.

The finding that nasal fractures were the most common injury at 54 per cent is similar to previous studies outside the UK. In 1972, Hall⁷ looked at all fractures of the facial skeleton in Melbourne, Australia, and Kaban et al.⁸, in 1977, studied these injuries in Boston, USA.

The preponderance of males with fractures of the facial skeleton of all types (the actual male:female ratio was 3:1 in this study), is in keeping with most earlier studies of facial fractures. The exception is mandibular fractures which have been variously reported from having an equal sex distribution^{8,9} to a male:female ratio 3.5:1¹⁰. This reflects differences in the cause of injury in those fractures sustained in RTA which in this study (with its male:female ratio of 3:1) mainly affected cyclists or pedestrians. However, in the studies where the mandibular fractures were mainly sustained by car passengers, the sex distribution was equal.

The age of peak incidence of facial fractures is difficult to compare as different studies have used different upper age limits for their samples. In this study the peak incidence was age 10 years, but all fracture types were more common in all those aged more than 5 years (only 17 per cent of fractures occurred in those aged 5 and below). This finding has previously been extensively reported in a review of both mandibular fractures and middle third facial fractures¹. The peak incidence at age 10 years is younger than the peak incidence of 13 years in a larger sample⁷, but a peak incidence at age 10 has also been reported for mandibular and middle third fractures in children¹¹.

The finding that fracture types were similar to those found in adults despite the differences in facial anatomy has been reported previously⁷. It has also been reported earlier that there is a higher incidence of fractures of the mandible involving the condylar head in children⁷, but there was no example of this type of fracture found in this series. However, the relatively high proportion of isolated orbital fractures in this series confirms a recently reported finding in the USA¹². There is a relatively higher proportion of dentoalveolar fractures in the children which has also previously been noted, but in this series they are equally distributed between the mandible and maxilla, whereas earlier studies have suggested that they usually occur in the anterior maxilla¹³. Overall, the results confirm that the proportions of the different types of facial fractures are different from adults.

The presence of associated injuries with facial fractures is well established and their presence has been recorded in up to 88 per cent of those with a facial fracture¹⁴. The associated injuries most commonly included head injury and facial lacerations but more severe injuries including multiple limb fractures and abdominal trauma requiring emergency laparotomy are also present. No chest or cervical spine injuries were recorded. The finding in this study that both the incidence and severity of associated injuries were more likely to be associated with mandibular fractures has previously been noted⁶. The presence of a wide range of associated injuries particularly with mandibular fractures in this series suggests that the treatment of patients with these injuries is best performed in

institutions where multidisciplinary management can be easily coordinated.

When trying to compare aetiology it has to be recognized that there is no universal classification. The aetiology of the fractures in this study shows falls, sporting accidents and RTA to be the major causes, in decreasing order of importance. Previous studies from outside the UK have identified RTA and in particular passengers of cars, as the major cause of facial fractures in children^{11,15} Further, it has been stated that in RTA children are much more commonly injured as passengers rather than pedestrians¹. This is in marked contrast to the findings of this study where RTA were only the third most common cause of these fractures, and in addition those involved in RTA were mainly pedestrians and cyclists.

The aetiology of facial injuries which had been sustained during a sporting event found a total of 13 sports of which golf, horse riding and soccer were the most common in this study. This compares with a finding of soccer, rugby and cricket as the most common cause of children's facial fractures in a UK study of sporting injuries^{15,16}. However, when the severity of facial injury was considered, this earlier study cited motor-cycle riding and horse riding as being associated with more severe injuries, a finding confirmed here.

Opportunities for prevention are limited when it is considered that little can be done to prevent a fall, that the number of sports producing injuries totalled 13 and that the overwhelming majority of RTA occurred to pedestrians or cyclists. Of the less frequently cited causes of injury, only assaults are readily preventable. Preventative measures such as early correction of malocclusion and the wearing of mouth guards during contact sport have been recommended⁷ but would have had little effect on this series of fractures. Also the results of this study suggest that introduction of rear seat belts in cars in the UK is unlikely to have much impact on the number of facial fractures in children (unlike the effect of front seat belts for adults). Other opportunities are few but it should have been possible for the motorcyclists to have been wearing helmets which covered the face, but the numbers involved are small.

The initial results of treatment were in most cases excellent. However the follow-up period for most patients was short and it may be that the late complication of altered growth, which had not been recorded in this series, has not yet had sufficient time to declare itself. In addition several cases with serious long-term sequelae are present in this series and these highlight the need of the clinician to be aware of these potential complications when making their initial assessment and planning management.

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I am grateful to all the consultants from the six different surgical specialties who co-operated and who made this

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Orbital floor fractures in young children

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SUMMARY. Isolated orbital floor fractures in children before the end of the 7th year of life are said to seldom occur. This is thought to be due to differences in anatomy from adults in that the maxillary sinus is developing and the orbit is still increasing in size.

Two cases of isolated orbital floor fractures in children aged less than 8 years are reported, their management discussed, and the literature reviewed.

CASE ONE

A 5-year-old girl fell 5 m from a playground slide on to the left side of her face, and was unconscious momentarily. She was immediately taken to an accident and emergency department and was admitted for neurological observations. Physical examination revealed a restriction of upward gaze. Radiology showed opacity of the rudimentary maxillary sinus on the affected side but the radiograph was reported as showing no fracture. The restricted eye movements persisted and ophthalmological assessment was sought. Computer tomography (CT) examination failed to demonstrate a fracture but with the symptoms persisting 7 weeks after the injury, orbital floor exploration was undertaken. A fracture only 1 mm across was found in the orbital floor with entrapped orbital fat and fascia extending down into the maxillary sinus. The orbital structures were released, and the defect, due to its small size, was repaired with pericranium.

18 months postoperatively she had a good cosmetic result with greatly improved eye movements (although there was still discernable limitation of vertical gaze).

CASE TWO

A 6-year-old boy fell 2 m from a climbing frame landing on to the left side of his face. He was taken immediately to casualty where he was admitted for neurological observations. Examination revealed a limitation of upward gaze but plain radiographs were reported as showing no fracture. After 1 week with no improvement in physical findings he was referred for ophthalmological examination. Radiographs were reviewed and an orbital floor fracture reported. Orbital floor exploration was undertaken 3 weeks after the injury. At operation, the associated soft tissue contents within the fracture were visualized before being returned to the orbit. The bony defect was then reconstructed using outer cranial table bone lined with pericranium.

1 year postoperatively he had a good cosmetic result with a much improved range of eye movements, although there was some limitation of movement on upward gaze.

DISCUSSION

Orbital floor fractures in children resulting in enophthalmos have been recognized for over one century (Lang, 1889), despite the fact that maxillofacial fractures in the paediatric age group are uncommon (Reil and Kranz, 1976; James, 1985). The term 'blow out' fractures of the orbit was first applied to those fractures of the orbital floor where the orbital rim is intact (Smith and Regan, 1957), but because of the anatomical differences in children below 8 years we prefer the term isolated orbital floor fractures in this group. Reports of isolated floor fractures in those aged below 8 years have been sporadic: one aged 11 months (Converse et al., 1967), one aged 5 years (Bartkowski et al., 1982) and one aged 7 years (Leitch et al., 1990). Other reports of these injuries involve children who are at least 8 years old, or the age is not stated (Thaller and Huang, 1992). This absence of reports is not surprising when it is remembered that the morphology of the orbit does not resemble that of an adult until 8 years of age and that the maxillary sinus is still developing (James, 1985).

Orbital floor fractures in children have usually been reported either with other paediatric maxillofacial injuries (Hall, 1972; Reil and Kranz, 1976; Anderson, 1995) or as a series of these injuries which includes adults (Converse et al., 1967; Bartkowski et al., 1982), or other types of orbital fractures (Kaban et al., 1977). This mixing of orbital fracture types has led to some apparently conflicting conclusions. It has been shown that when all types of orbital fractures in children are considered they are found to be relatively common in comparison with adults (Posnick et al., 1993). However, Posnick et al. (1993) demonstrated within this group that isolated orbital floor injuries are rare (less than 10% of all paediatric orbital fractures). This



Fig. 1 – Patient looking upwards with failure of the affected left eye to elevate (case 2).

contrasts with the findings of a series of 'blow out' orbital fractures involving both children and adults where it was reported that 38% occurred in children (Bartkowski and Krzystkova, 1982). However, closer inspection revealed that the average age of the children in this series was 11.2 years and no case was younger than 5 years of age. Overall, this conflicting data has resulted in differences in the reported incidence of these injuries in children as a whole, but there can be little doubt that they are very rare in children aged below 8 years of age.

In adults, both the anatomical basis and the mechanism of the injury have been described in detail (Jones and Evans, 1967; Fujino and Makino, 1980). However, we could find no references to data pertaining to either of these in the immature facial skeleton. Undoubtedly, despite the small size of the maxillary antrum, escape of orbital contents through the orbital floor may still occur, and considerable local force would be required to produce the injury. As considerable force is required it is curious that in neither of our two cases were the injuries sustained as a result of a traffic accident, which has been shown to be the commonest cause of all paediatric maxillofacial fractures in reports from the following countries: US (McGraw and Cole, 1990), Australia (Hall, 1972), Greece (Stylogianni et al., 1991) and Germany (Reil and Kranz, 1976).

Diagnosis is by a combination of clinical examination (Fig. 1), radiography (Fig. 2) and CT scan. It is interesting to note that in all of these cases the plain radiographs were reported as normal but the fracture was subsequently reported as present on review. This highlights the difficulty for radiologists to recognize this rare condition in the immature facial skeleton.

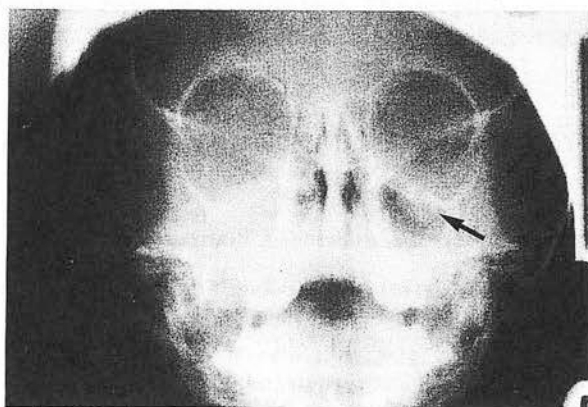


Fig. 2 – Radiograph showing 'tear drop' sign on affected left side (arrow), (Case 2).

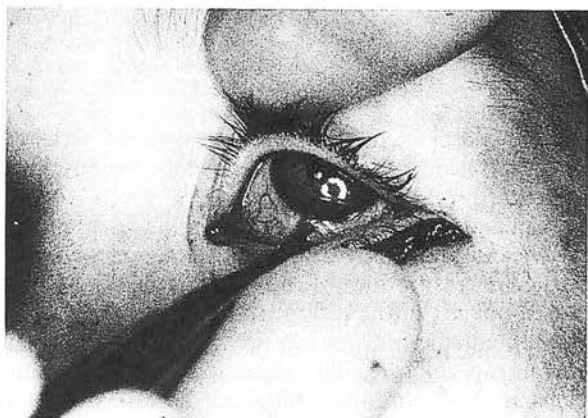


Fig. 3 – Fixed duction test which is positive on the left side (case 2).

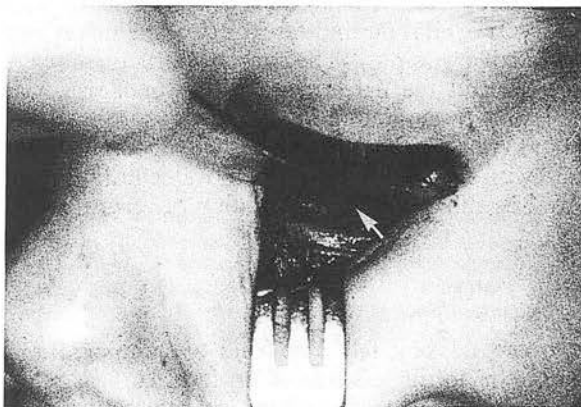


Fig. 4 – Orbital floor at exploration showing fracture arrowed (case 2).

The limited value of plain radiographs in assessing these injuries has previously been noted (Ilankovan et al., 1991).

The treatment of our cases involved operative intervention to release the entrapped tissue. A forced duction test was performed to detect limitation of movement of the eye (Fig. 3) after which we proceeded to open exploration of the orbital floor to visualize the defect (Fig. 4), and to release entrapped tissue. It has

been recommended that in children surgical intervention should be performed within a few days of injury in an attempt to reduce postoperative enophthalmos (Mulliken et al., 1977). However, in adults it has been reported that conservative management with orthoptic exercises has improved diplopia better than surgical exploration (Emery et al., 1971). Consequently, it has been suggested that the condition is often better managed by conservative treatment (Koornneef, 1982; Everhard-Halm et al., 1991).

Our cases highlight the difficulty in managing to operate early due to the problem of establishing the diagnosis. Others have suggested that exploration should be attempted via the maxillary sinus (Bales et al., 1972). Antral packing is not recommended as the maxillary sinus is too small, a point which has been made previously (James, 1985). Attempts should be made to preserve as much of any comminuted bone as possible as this is likely to heal. However, a bony defect may result in the orbital structures requiring support. Many materials have been recommended for repairing the defect including both autogenous and alloplastic materials (Morain et al., 1987). However, we prefer pericranium for a fissure fracture, and for a larger defect use pericranium with overlying cranial bone, as these cases illustrate. The use of pericranial free grafts for coatings, augmentations and suspensions in the face have been previously reported (Powell and Riley, 1989). This has the advantage in that it allows the orbital soft tissues to glide over the repair. It also overcomes the problems of migration of a prosthetic implant (Wilkins and Havens, 1982). However, we note the absence of reported long-term complications using this in a series of children of unreported ages (Mulliken et al., 1977).

Early and late complications of the condition and its treatment in adults and children are recognized (Wilkins and Havens, 1982; Rathbun, 1984; Ousterhout and Vargervik, 1987), although none have occurred in our cases. Reported late complications resulting from injuries sustained in childhood (although not necessarily before 8 years of age) include: post-traumatic enophthalmos (Mulliken et al., 1977); diplopia (Leitch et al., 1990); chocolate cyst formation, which has been reported by Sutula et al. (1991) as occurring 21 years after injury; and finally, in association with other midfacial fractures, subsequent development of midface hypoplasia (Ousterhout and Vargervik, 1977). It has been suggested that there is an increased risk of diplopia following orbital floor fracture in younger patients (Waddell et al., 1982). The latter series does not include any children younger than 8 years of age and the findings were not reproduced in a second study if the time from injury to operation did not exceed 2 months (Leitch et al., 1990). The evidence for the increased risk of diplopia in these young children therefore remains tenuous. However, because of the known risk of developing complications we support the recommendation for long-term follow-up of these young patients (Mulliken et al., 1977).

In conclusion we report two cases of this rare injury in young children. The difficulties in radiographic

diagnosis have been described. The surgical management has been discussed. Finally, long-term follow-up of this group is recommended.

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Case Report

Hyperostosis as a late sequel of parasymphyseal mandibular fractures in 2 children

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SUMMARY. Background: The potential problem of growth anomalies affecting a mandible following a fracture of a mandibular condyle in childhood is well established. However, there have been no previous reports of this phenomenon affecting other fracture sites in the mandible. Patients: Two patients who had parasymphyseal fractures treated in childhood presented at skeletal maturity with hyperostosis at the fracture site, producing chin asymmetry in their teens. Results: In both cases the hyperostosis produced significant chin asymmetry without disturbance of the occlusion. Both patients were managed with corrective genioplasty. Conclusion: These cases reinforce the previous recommendations regarding the need for long-term follow-up of children who sustain facial fractures of the mandible, and that the protocol should be expanded to include parasymphyseal fractures as well as fractures of the condyle. © 2005 European Association for Cranio-Maxillofacial Surgery

Keywords: fractured mandible; late complication

INTRODUCTION

Facial fractures in children are uncommon, but within this population fractures affecting the mandible are relatively common. It is well established that there is a potential problem of subsequent growth anomalies following a fracture of a mandibular condyle in childhood. However, there have been no previous reports of localized bone growth disturbances affecting the mandible at other sites in the literature.

PATIENTS

Case 1

A 4-year-old girl was a back seat passenger in a car involved in a high speed motor vehicle accident. She sustained a fracture to the mandible in the right parasymphyseal region (Fig. 1). In addition she sustained a mid-face fracture affecting the ipsilateral maxilla and malar bone. She was taken to theatre where the mandibular fracture was treated by reduction and internal fixation using a plate and screws.

She was kept under annual review and by 7 years post-injury was noted to be developing asymmetry of the chin. Fourteen years after her fracture she requested surgical correction to improve her facial

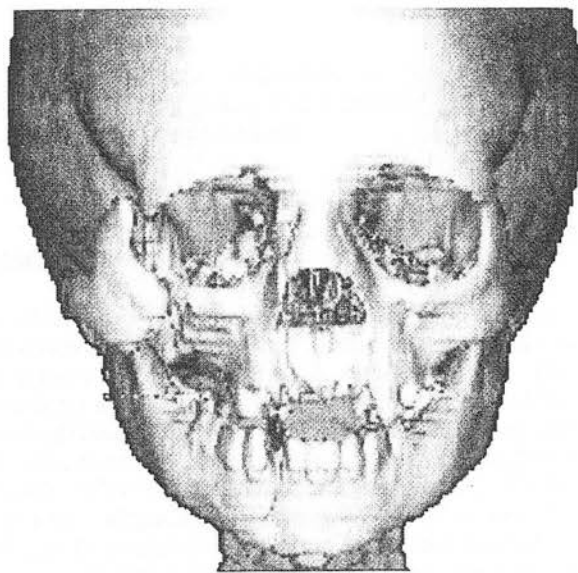


Fig. 1 – 3D CT scan of patient 1 pre-operatively, demonstrating the parasymphyseal fracture of the mandible and the mid-face fracture.

appearance. She had no TM joint symptoms at this time and her clinical appearance demonstrated asymmetry with her chin point towards the side of the fracture (Fig. 2a). Intraoral examination revealed an Angle's class I occlusion with no occlusal cant. A PA mandible radiograph confirmed the presence of localized hyperostosis at the site of the previous fracture (Fig. 2b).

*No financial support was provided to support this study.

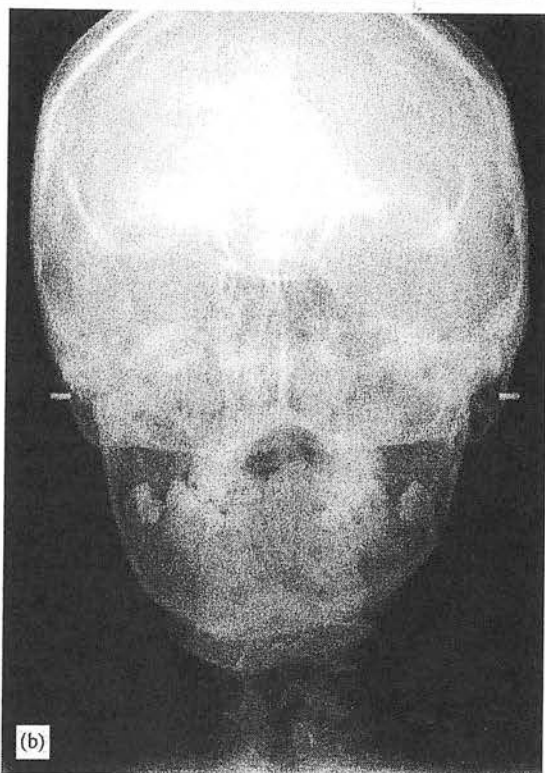


Fig. 2 – (a) Clinical appearances of patient 1, mandibular asymmetry and deviation of chin point to the side of former fracture (age 15 years). (b) Radiograph PA of patient 1 age 15 years, hyperostosis at former fracture site of mandible.

localized hyperostosis at the site of the previous fracture (Fig. 2b).

A genioplasty was performed to centralize her chin and 6 months later she was pleased with her appearance.

Case 2

A 5-year-old boy was a back seat passenger in a car involved in a motor vehicle accident. As a result he sustained a fracture of the mandible in the right parasymphiseal region. His fracture was displaced and produced a disturbance of his dental occlusion. He was taken to theatre and the fracture reduced using arch bars and intermaxillary fixation. Three weeks later the intermaxillary fixation was removed.

His immediate post-operative course was uneventful and his appearance satisfactory, apart from an immature scar overlying the chin. He was kept under review during childhood, but after 5 years it became apparent that he was developing mild asymmetry of his lower chin. This was noted to slowly increase with time and by 14 years following the fracture he requested further treatment to improve the facial asymmetry (Fig. 3a). He had no TMJ symptoms and on examination had no occlusal cant. The PA mandible radiographs confirmed the presence of localized hyperostosis at the site of the previous fracture (Fig. 3b).

He subsequently underwent a genioplasty to centralize the chin point, and 6 months later he was satisfied with his appearance.

DISCUSSION

Fractures of the facial skeleton in children are uncommon, but when they occur the mandible is commonly affected (Hall, 1972; Anderson, 1995). The long-term consequences of fractures of the mandible are well recognized when the condyle is fractured and so is the potential for its subsequent effects on facial growth (Rowe, 1969; Demianczuk et al., 1999). However, alteration in condylar growth can be difficult to predict in individual cases and this led to the recommendation that long-term follow-up should be undertaken in such cases (Hovinga et al., 1999).

The cases presented in this series both appear to have undergone localized hyperostosis following the fracture, producing the chin asymmetry. A similar localized phenomenon has been reported at another site in the mandible, following condylar fractures (Lund, 1974). But there is no previous reference to it occurring in the parasymphiseal region (or indeed any other region) of the mandible. However, it has been recognized that there may be growth disturbances affecting other sites of the facial skeleton. Following mid-face fractures in childhood subse-

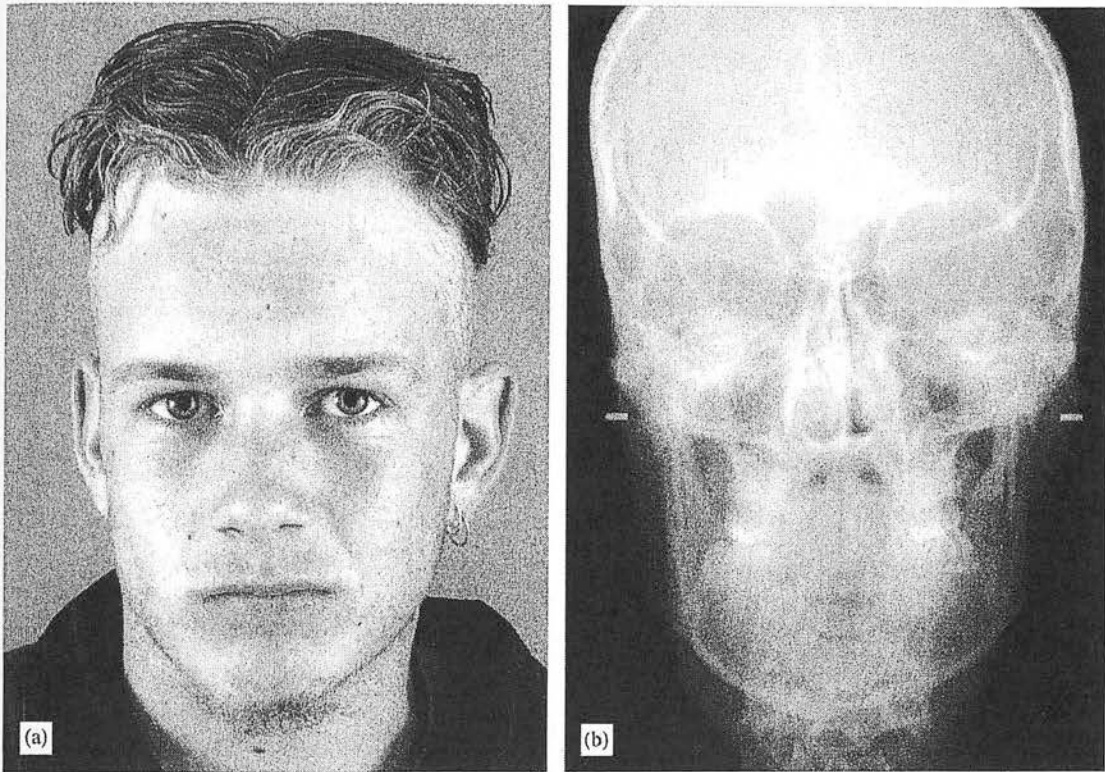


Fig. 3 – (a) Clinical appearances of patient 2, mandibular asymmetry and deviation of chin point to the side of former fracture (age 17 years). (b) PA radiograph of patient 2 age 17 years, hyperostosis at former fracture site of mandible.

quent growth retardation has been reported (Ousterhout and Vargervik, 1987).

Unfortunately there was nothing notable in the management of these cases to alert a clinician to the likelihood of subsequent localized overgrowth (apart from the possibility that it may be significant that these injuries were the result of high-energy impacts). The absence of a clearly identifiable factor in the aetiology of this localized hyperostosis means that the development of this late complication remains unpredictable.

CONCLUSION

Two cases of late complications of mandibular fractures of the parasymphysal region occurring in children are presented. These cases reinforce the previous recommendations regarding long-term follow-up of children who sustain facial fractures of the mandible. The protocol should be expanded to include mandibular fractures at this site as well as fractures of the condyle.

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CASE REPORT:

Facial Fractures as a Result of Dog Bite: A Case Report

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Abstract:

Background and Objectives. Dog bites in the face are common injuries in children; usually, they result only in soft tissue injury. Occasionally, injuries to the facial skeleton that result in fractures have been reported. The authors report a case — which they believe is unique, with soft tissue injuries and fractures of the facial skeleton at two sites — and discuss the management of this and related cases.

Methods and Materials. The injuries involved deep lacerations of soft tissue, the facial skeleton, and destruction of the inferior canaliculus of the nasolacrimal duct. Intraoperative radiographs and computerized tomography (CT) scans disclosed fractures in the right infraorbital region. The facial wounds were debrided and sutured, and antibiotics and blood transfusion were administered. Open reduction and fixation with resorbable miniplates and screws were performed 5 days subsequently. Medial canthopexy was used. The malar fracture was treated conservatively; the nasolacrimal duct was beyond reconstruction.

Results and/or Conclusions. Nine months postoperatively, there were no complications. The scars were settling, and the patient was maintained under a long-term review.

Key Words: Facial fractures, dog bite, and resorbable miniplates and screws.

CASE REPORT

A 1-year-old child was referred on an emergency basis after having been bitten 4 hours earlier by the family's pet rottweiler in the face and both orbits, sustaining deep lacerations (Figures 1 and 2). The child was transferred from home to the authors' clinic (a distance of 140 miles), and the wounds were debrided, irrigated, and explored under general anesthesia 6 hours following the injury. It became evident during the exploration that the injuries were more extensive, involving not only destruction of the inferior canaliculus of the nasolacrimal duct but the facial skeleton as well.

Intraoperative facial radiographs confirmed that fractures were present in the right infraorbital region. The frontal process of the left malar was indistinct, suggesting the possibility of



Figure 1. Preoperative right lateral view of a 1-year-old child who sustained dog bite injuries to the midface around the infraorbital and nasal region of the right orbit.

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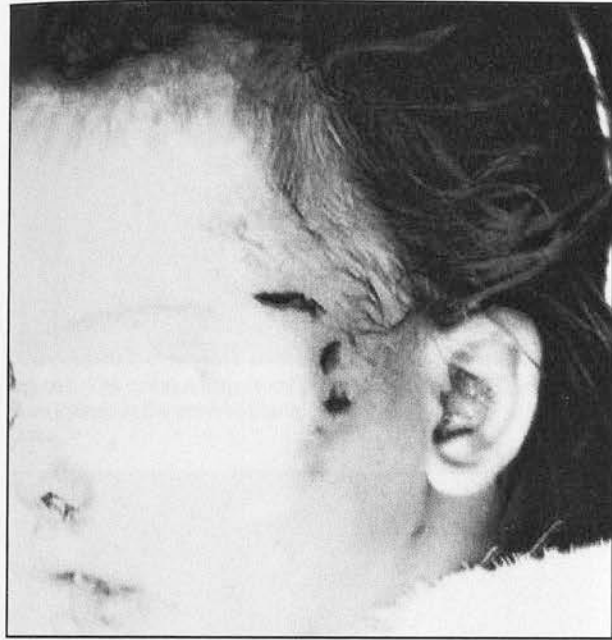


Figure 2. Preoperative left lateral view of the injuries sustained around the lateral aspect of the left orbit.

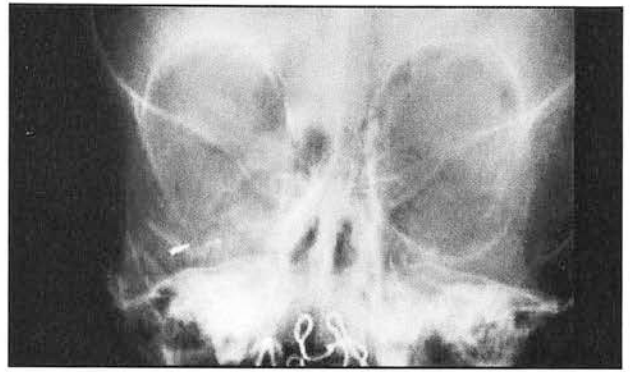


Figure 3. The radiograph reveals a displaced fracture of the right infraorbital region (arrowed) and the absence of a clear outline of the left lateral orbital wall.



Figure 4. The CT scan demonstrates the full extent of the displacement of the right medial orbital fractures in the transverse plane.

another fracture (Figure 3). Therefore, prior to undertaking the definitive management, further clarification of the extent of the fractures was thought to be appropriate. The facial wounds were cleaned, debrided, disinfected, and sutured. Antibiotic treatment was commenced, while a simultaneous blood transfusion was performed.

A computerized tomography (CT) scan was performed on the following day to ascertain the exact nature and extent of the fractures. The scan revealed the presence of displaced fractures of the right nasoethmoidal complex (Figures 4 and 5) and a minimally displaced fracture of the left malar bone.

The facial swelling was allowed to subside for 5 days, at which time a second procedure was undertaken. The right infraorbital wounds were reopened to allow access. The examination revealed no evidence of infection. The displaced fractures of the nasoethmoidal complex were treated by open reduction and fixation, using resorbable miniplates and screws (Forth Medical Ltd, Newbury, UK) (Figure 6).

A medial canthopexy was undertaken to reestablish the position of the lids, since both the anterior and posterior limbs of the medial canthal ligament were damaged as part of the original injury, rather than during the surgical repair. The nasolacrimal duct was beyond reconstruction. The left malar fracture was treated conservatively. The lower and common canaliculi were both damaged during the injury. The damage resulted in the destruction of these structures for much of their length, so that reconstruction in a child of this age was impossible. As a result, the patient had difficulty with recurrent epiphora immediately postoperatively. Four days postsurgery, the patient was well enough to be discharged and has since been maintained under a regular review.

Eight months postoperatively, the recurrent epiphora had resolved spontaneously. No further surgery to the lacrimal system is planned in the absence of symptoms. Nine months post-surgery, the patient is well. The scars are settling (Figure 7), and no complications have resulted from the use of fixation plates. The patient is maintained under long-term review to ensure that facial growth is occurring normally.

DISCUSSION

Fractures of the facial skeleton in young children are not common, but when they do occur they are usually the result of traffic accidents, falls, or sporting injuries.¹ Conversely, dog bites to the faces of children are seen commonly, with the more severe injuries occurring in children under 2 years of age.² Associated injuries with dog bites may include disruption of lacrimal canaliculi³; damage to levator muscle of eye, producing ptosis³; and damage to the nasal cartilage.⁴ However, very few cases have been reported of facial fractures in children resulting from a dog bite.^{3,5} In these cases, the fractures identified have been isolated injuries, either to the malar or the nasal bones.

There are two aspects in the management of these injuries — the treatment of the potentially infected tissues⁶ and reconstruction of the skeletal and soft tissues. The priority in treatment planning is to first address the high risk of infection following a dog bite. This requires an early wound antisepsis to prevent infection, and its importance cannot be overstated.³

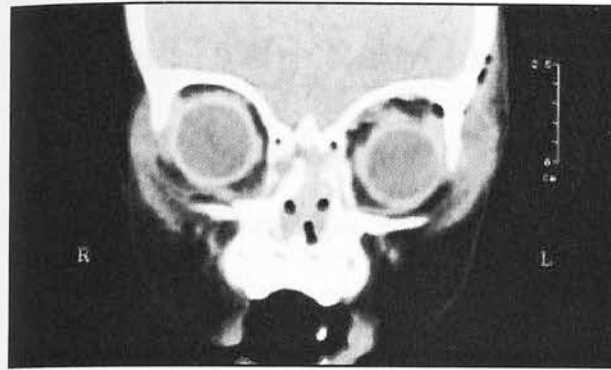


Figure 5. The computerized tomography scan demonstrates the same injuries in the coronal plane.



Figure 6. Intraoperative view of the right nasoethmoidal fracture, reduced and fixed with a resorbable plate.

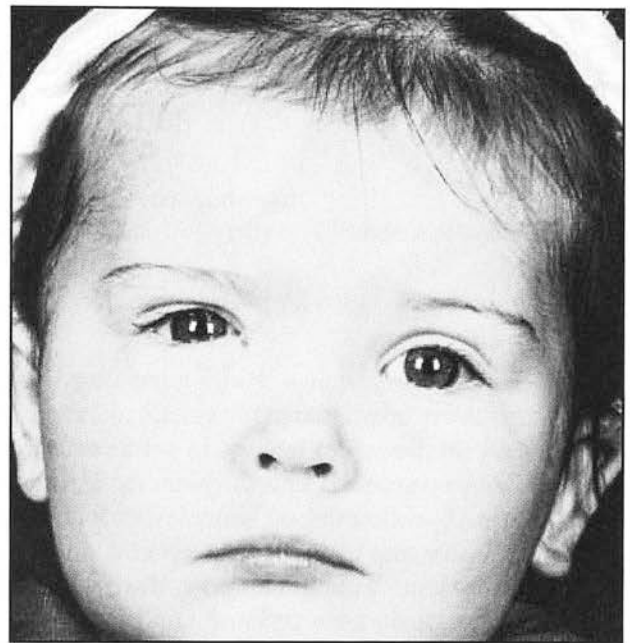


Figure 7. Nine-month postoperative appearance of the patient.

Topical antibiotics are used commonly, although their role is uncertain if early wound debridement is undertaken. However, in cases where a delay has taken place prior to the treatment, or in children who have undergone previous splenectomy and are at risk for life-threatening fulminating septicemia,⁷ a penicillinase-resistant penicillin or cephalosporin is recommended to provide an adequate spectrum for treatment of the canine oral flora.⁸ In addition to infection resulting from the normal canine microbiologic flora, the possibility of transmission of rabies or tetanus should also be considered, and tetanus prophylaxis should be administered, if required.

The use of resorbable plates to hold the reduced fracture in the correct position has the obvious advantage that no additional procedure is necessary for their removal.⁹ In children with active sutures, the resorbable fixation materials may have an additional advantage when compared to the conventional titanium plates. It has been demonstrated in animal studies that when metal plates are positioned across growing sutures, the plates may retard the skeletal growth.^{10,11}

CONCLUSION

A case of an unusual injury has been presented, involving facial skeletal fractures and soft tissue injuries resulting from a dog bite. Details of the management of such injuries have been discussed. This case again highlights the need for a high index of suspicion of facial fractures in children who are victims of

dog bites to the midface region and who are under 2 years of age.³ Consequently, the use of radiographs should be considered as a part of the early assessment to identify any damage to the underlying skeleton in such children, even when the facial fractures are not clinically apparent, as they were in this case. It must be remembered that any delay should be avoided in treating the microbiologically contaminated tissues and in performing the surgery to reduce the fractures. Since the fractures heal rather quickly in children, a prompt treatment will prevent the development of complications, avoid any additional difficulties in reduction and fixation, and present the most optimal treatment results.

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A Pitfall in the Radiological Diagnosis of Paediatric Mandibular Condylar Fractures

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Abstract

Horizontal paediatric condylar fractures are a relatively common injury, which can have lifelong consequences, if not managed properly at initial presentation. Successful management relies on accurate diagnosis of the fracture, which leads to the determination of optimal treatment. We present a case of a fractured mandible with vertical fractures of both condyles and the parasymphysis in a child, which was difficult to diagnose using conventional radiological interpretation. The initial radiographs did not fully reveal the nature of the condylar trauma and computed tomography was necessary to clarify the extent of the injuries. Management was a combination of open reduction internal fixation for the parasymphyseal fracture, and orthodontic brackets were cemented to the teeth to allow elastic traction and conservative management of the condylar fractures, which involved both temporomandibular joints. The patient made an unremarkable postoperative recovery and had regained excellent range of motion within 2 weeks of surgery. This case highlights a diagnostic difficulty using orthopantograms and mandible radiographs, and illustrates a useful management strategy for bilateral vertical fractures of the mandibular condyles.

Key words: Diagnosis, Internal fracture fixation, Mandibular condyle, Reconstructive surgical procedures, Temporomandibular joint

Introduction

Paediatric facial fractures are not common, but several epidemiological reviews have identified that mandibular condylar fractures are a relatively common site of injury.¹⁻⁴ Conservative management of such fractures has been shown to be successful,⁵⁻⁸ although open reduction has also been recommended.⁹ Currently, many surgeons manage minimally displaced condylar fractures of the neck without surgery, allowing the condylar head to remodel itself naturally. The following case outlines the management of a usual case of bilateral vertical fractures of the condylar heads, and illustrates the difficulty in diagnosis with conventional radiography.

Case Report

A 9-year-old girl, who was previously fit and well, fell from a height of about 4 feet striking her chin on a concrete surface. On initial presentation she was found to have a

3-cm laceration on her chin as well as swelling and tenderness along the left side of her mandible. She also had an obvious malocclusion and was only comfortable with her mouth in an open state (Figure 1).



Figure 1. Preoperative photograph of the patient showing soft tissue swelling and mandibular deviation on opening.

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Palpation over the temporomandibular joints revealed tenderness bilaterally. Plain radiographs were taken, including an orthopantomogram and facial views. These films demonstrated a displaced left parasymphysal fracture on the left, and were suspicious for a left condylar fracture as well (Figures 2a and 2b).

The radiologist was unable to determine the true nature of the condylar fracture, and therefore computed tomography (CT) was performed, which demonstrated a sagittal fracture in the left condylar head with medial displacement of the fractured medial segment. In addition, an undisplaced fracture was noted in the right condylar head. The ramus appeared to have maintained normal height on both sides (Figure 3).

A treatment plan was formulated in which the comminuted parasymphysal fracture was treated with open reduction internal fixation using 2 titanium plates placed below the level of the developing teeth and tooth roots. Monocortical screws were used on both plates. To treat the condylar fractures, orthodontic brackets were placed on molars on each side to be used for rubber bands (Figures 4a and 4b).

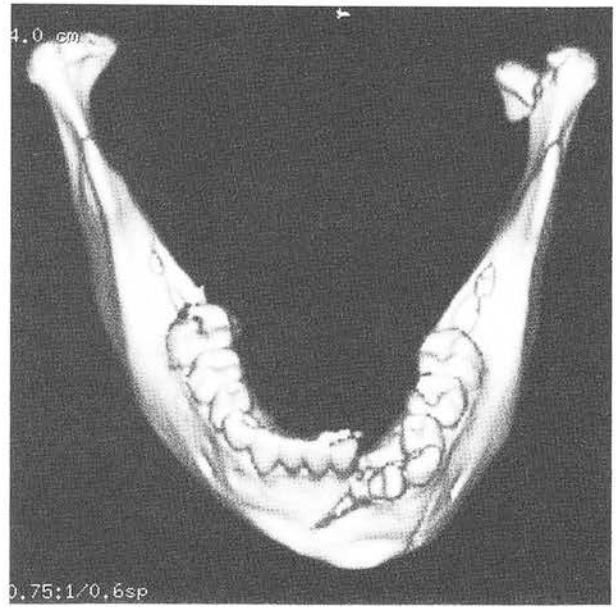


Figure 3. Preoperative 3-dimensional reconstruction of mandible demonstrating condylar injuries (left displaced, right undisplaced).

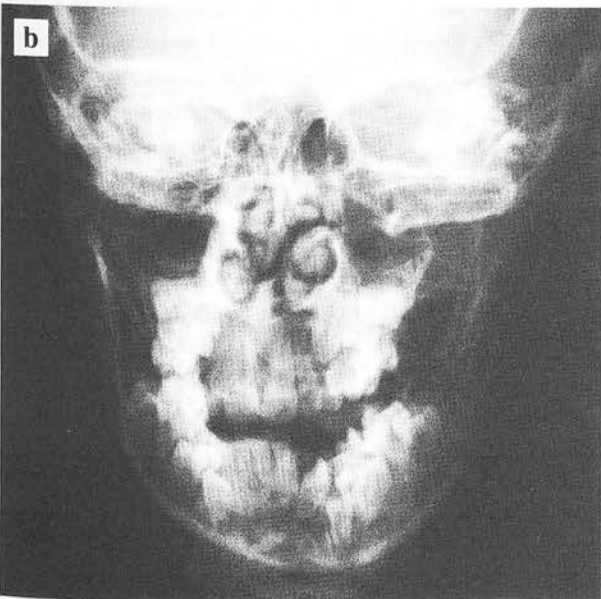
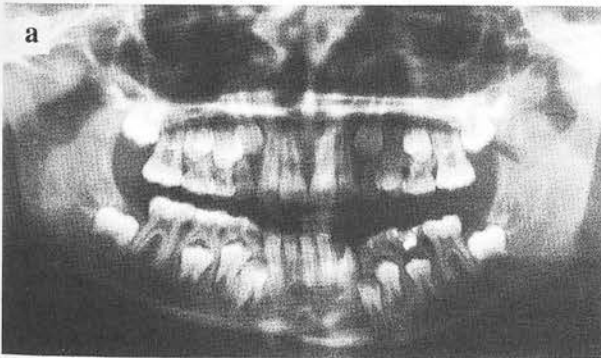


Figure 2. (a) Preoperative orthopantomogram. (b) Preoperative anterior-posterior facial radiograph.

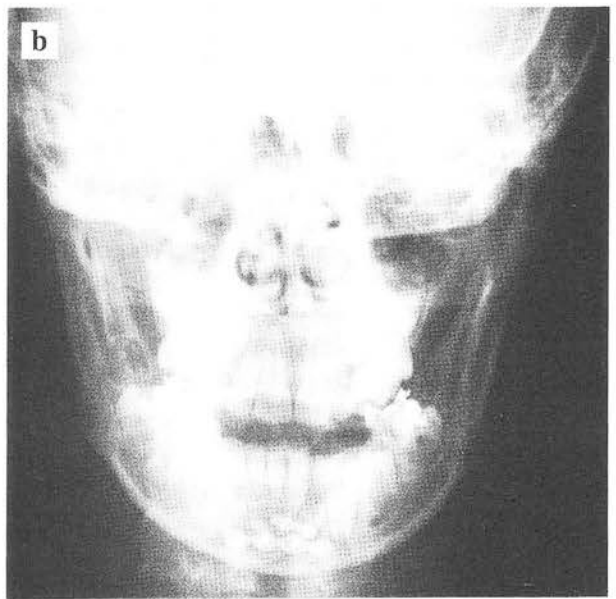
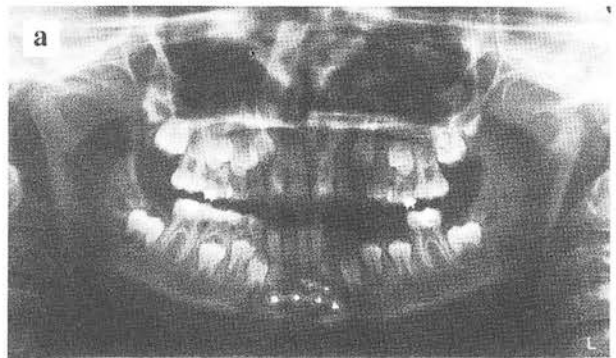


Figure 4. (a) Postoperative orthopantomogram. (b) Postoperative anterior-posterior radiograph.



Figure 5. (a) Postoperative photograph taken at 6 months showing with mouth opening, but with no mandibular deviation. (b) Postoperative photograph taken at 6 months showing the occlusion.

These bands served to guide the patient back into normal occlusion and provide relief of muscular spasm during the first 2 postoperative weeks. The patient was encouraged to begin jaw motion as soon as possible to prevent ankylosis. Within 1 week of surgery she was comfortably moving her jaw and was able to achieve an interincisal opening of 25 mm. She was kept on a soft diet for 4 weeks. Follow-up performed 6 months after surgery revealed a satisfactory occlusion with the lower left canine and premolars erupting and normal mandibular opening (Figures 5a and 5b).

Discussion

Paediatric mandible fractures can often be difficult to assess and repair due to the developing permanent dentition. Previously, it has been shown that when comparing CT versus orthopantomogram, there is a clear advantage for CT scans in diagnostic accuracy with respect to condylar fractures¹⁰ and our experience with this case reinforces this. However, it is impractical for all cases with mandibular condylar fractures to undergo CT evaluation, and this requires careful evaluation by clinicians who are aware of the potential for unusual fracture patterns. In this case, the clinical findings of temporomandibular joint tenderness with coexisting uncertainty in interpreting the radiological evaluation were thought to be significant clues.

Our management strategy for paediatric mandible fractures takes into account the location and severity of the fractures along with the amount of malocclusion. With regard to condylar fractures we believe that early range of motion exercises are of paramount importance in avoiding ankylosis. It has also been our experience that the use of rubber bands for assistance in maintaining occlusion and providing relief of masseteric swelling and spasm has been a helpful adjunct to our management plans. Conservative management has been supported by some authors,^{7,8} even in the presence of severe dislocation.¹¹

Management of the displaced parasymphiseal fracture is based on using 2 plates fixed with monocortical screws to provide stable fixation without damaging the forming tooth structures or roots. Alternatives to the second plate, such as bridal wires and splints are also useful⁸ and may be necessary if there is no room for a second plate. Long-term follow-up for fractures such as these is extremely important. Condylar fractures may result in long-term growth disturbances and parasymphiseal fractures may result in contour deformities due to hyperostosis.¹²⁻¹⁴

In conclusion, this case represents an injury sustained from a traumatic blow to the chin. Condylar fractures should be suspected in such injuries¹⁵ and were indeed found in this case. Furthermore, such fractures are best seen using a CT scan. Conservative management of such injuries has been found to be successful and should be the first choice

for non-displaced condylar fractures or those that do not significantly affect occlusion or mandibular height and symmetry.

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Clinical observation

Medial wall blow out fractures in children

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ABSTRACT

Background: Fractures of the facial skeleton in children are relatively uncommon in comparison with adults. Within the orbital region fractures are recognised to be rare due the timing of development of the maxillary and frontal sinuses which are not fully developed until 12 years of age. We have recently seen several cases of medial wall fractures in children, which according to the existing literature should be exceptional. This prompted a review of the unit database to clarify the incidence and identify common aetiological factors for these injuries.

Method: Retrospective case note review using the ACFU database.

Results: Six cases were identified with a most cases occurring in males. The aetiology was found to be variable in each case with no identifiable pattern and the subsequent management reviewed.

Conclusion: These six cases highlight that this pattern of fracture can occur more commonly than the existing literature suggests and this should be remembered by those who treat facial trauma in children.

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1. Background

Fractures of the facial skeleton in children are relatively uncommon in comparison with adults [1]. Even within the paediatric population orbital fractures are relatively rare with fractures of the nasal bones reported as the commonest site for injury [1]. When fractures do occur in the immature facial skeleton orbital fracture patterns can be different to those in adults [2], because the maxillary sinuses are not fully developed until age 12 years. Fractures of the orbit in children can also affect the thin walled orbital roof, with orbital floor fractures exceptional in infancy, but becoming relatively more common with increasing age as skeletal maturity approaches [2].

However, we have recently been surprised given the apparent rarity of orbital injuries in children to manage two patients with fractures in the immature facial skeleton at another site within the orbit, the medial wall, a site with only occasional reports of fractures in children [3]. Review of case notes identified a further four cases over a 5-year period highlights that these patterns of fracture can occur in children and suggests that they may be more common than the existing literature would indicate.

We report these two cases and review the earlier four cases to identifying common features which could aid clinicians in recognising such injuries. The management of these injuries is discussed and we recommend that any patients are optimally managed

in centres where co-ordinated multidisciplinary management is available.

2. Case reports

2.1. Case 1

A 9-year-old girl was sitting at her computer desk when she was suddenly startled resulting in a reflex jump and her hitting her orbit with her knee. She had no symptoms other than mild swelling of her eye until she blew her nose a day later when her eye became very swollen. She attended her general practitioner and was referred for specialist opinion. After a 3D CT scan was obtained a medial blow out fracture was identified. This was identified to be approx. 5 mm × 5 mm and after ophthalmological opinion confirmed no ocular injury was treated conservatively. After 12 months she was reviewed and had no further symptoms.

2.2. Case 2

An 11-year-old boy was in an athletics competition and while landing in the high-jump managed to hit his knee into his eye. He had a swollen tender eye was referred for specialist opinion. After a 3D CT scan it was noted that he had sustained a medial blow-out fracture of his orbit approx. 10 mm × 8 mm in diameter. He was noted to have enophthalmos and limitation of lateral field of gaze, but no other ocular injury. He was treated surgically with access to the orbit via a coronal scalp flap. The orbital contents were returned to orbit and reconstruction of the medial orbital wall undertaken

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Table 1
Medial orbital fracture cases.

Age in years	Gender	Injury mechanism
7	Male	Fall
9 (Case 1)	Female	Knee
11 (Case 2)	Male	Sport (athletics)
13	Male	MVA (car driver)
13	Male	Bicycle
14	Male	Assault (punch)

using a thin cortical bone graft from the inner aspect of the iliac crest.

He made an uneventful post-op recovery and was discharged home 2 days later with a full range of eye movements in all positions of gaze. Six months later he has made a full recovery, with no enophthalmos or diplopia, and is back high jumping!

The two cases presented here along with the four previous cases are summarised in Table 1.

3. Discussion

It is notable in this series of six, that all except one case occurred in males, in keeping with other fractures of the immature facial skeleton [1]. These cases have sustained fractures of the medial orbital wall by a range of different mechanisms. This highlights the difficulty in suspecting such an injury from the history alone. The subsequent clinical examination and signs may include initial periorbital swelling, subconjunctival haemorrhage and later enophthalmos [4], but these can be subtle as highlighted in Figs. 1 and 2, which makes the diagnosis difficult for the clinician. The difficulty in establishing the diagnosis is further complicated because it is also difficult to diagnose these fractures on plain radiographs and while high resolution CT scans are the investigation of choice, they may be difficult to obtain outside specialist centres, so good radiology is essential.

Fractures of the orbits have been reported to be rare injuries in children [1]. Although growth of the orbits is almost complete by 4 years of age [5], the fracture patterns can be different to the adult population due to the differences in the anatomy of the surrounding peri-orbital structures in the immature facial skeleton.



Fig. 1. Case 1 at presentation with left periorbital swelling.



Fig. 2. Case 2 at presentation with mild periorbital swelling.

This includes the relative thickness of the orbital floor because the maxillary sinuses only become fully developed at age 12 years of age, explaining why orbital floor fractures below this age are rare [2]. In contrast fractures of the orbital roof are relatively common in this the younger paediatric population, due to the relatively prominent position of the frontal bone in a child's face. However fractures elsewhere in the orbits in children according to the existing literature are exceptional [3], and even in adults medial orbital wall fractures are relatively uncommon.

In children at this site there are important regional anatomical differences in skeletal maturity in comparison with other sites within the orbit (the roof and the floor) because the adjacent anatomical structures, the ethmoid air cells, are already present in similar arrangement to the mature adult skeleton by 4 years of age [6]. This anatomical arrangement resulting in thin bone lining the orbit, resembles that seen at skeletal maturity, results in any fractures of the medial orbital wall occurring in children older than 4 years demonstrating an adult type fracture pattern. This was seen in all of the radiological studies of these cases and highlighted in Figs. 3 and 4 which demonstrate fractures.

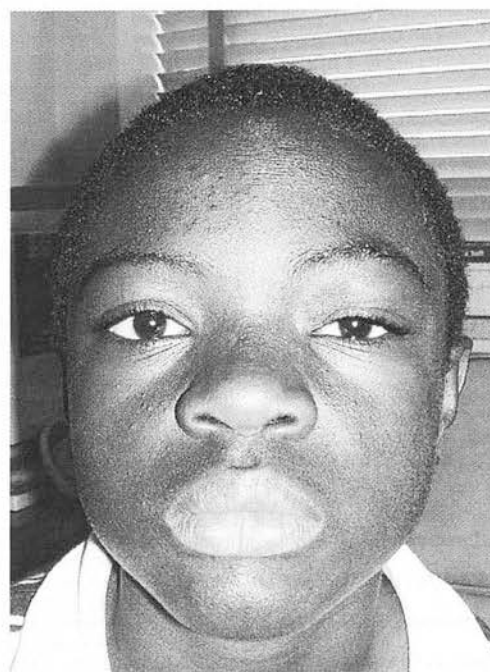


Fig. 3. Case 1 CT scan showing medial wall fracture, and surgical emphysema.

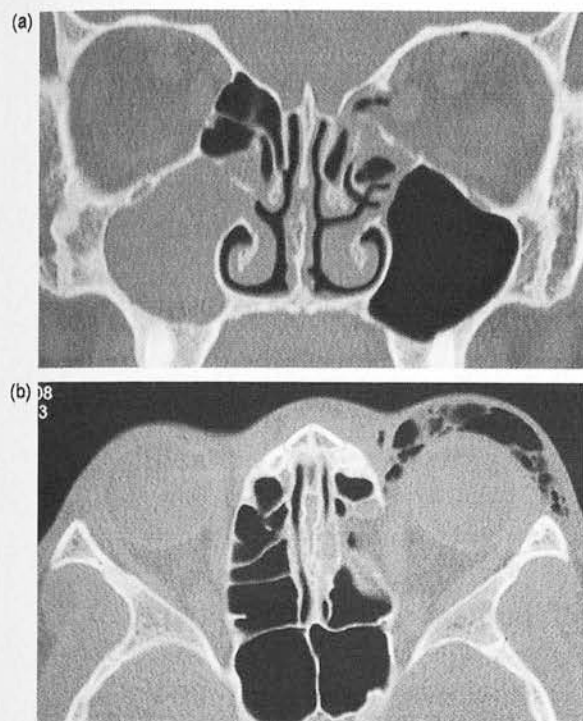


Fig. 4. Case 2 pre-op CT scan showing fracture. (a) Coronal view, (b) horizontal view.

The management of these fractures can be conservative for small defects with no enophthalmos or limitations of extra-ocular muscle function as in Case 1. These defects can be measured and those 5 mm × 5 mm are managed with 5 days antibiotics and advised to avoid blowing their nose to prevent surgical emphysema. However, defects with enophthalmos due to herniation of orbital contents into the nasal cavity or entrapment of the medial rectus muscle [7] require return of the soft tissues to their correct place and reconstruction of the orbital wall with iliac crest onlay bone graft to prevent relapse as in Case 3. A coronal scalp flap allows good visualisation of the fracture site, although repair via a medial canthus [8] or a transcuruncular approach have been advocated [9] and even more recently endoscopic repair has been reported in adults [10] (Figs. 5 and 6).

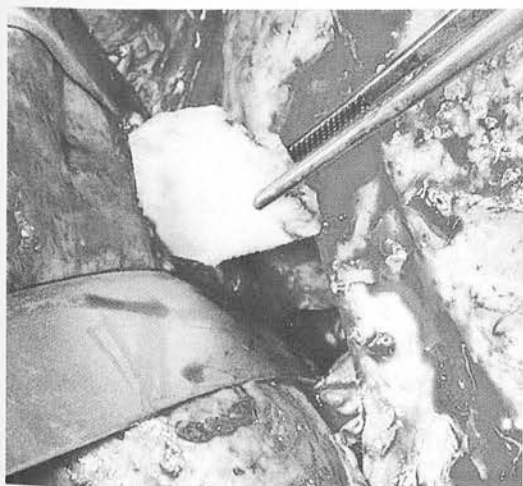


Fig. 5. Case 2 intra-operative view demonstrating fracture after the orbital contents have been released, with iliac crest bone graft to be onlayed over the fracture.

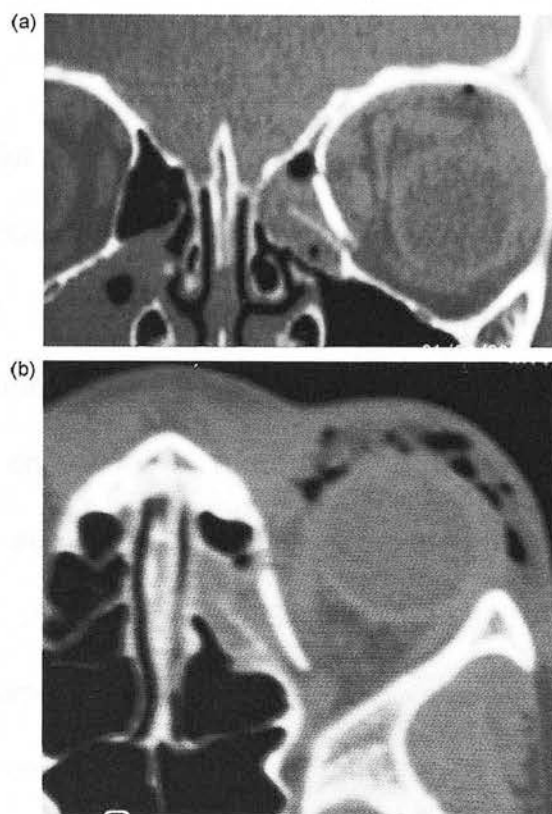


Fig. 6. Case 2 post-op CT scan showing graft in position. (a) Coronal view, (b) horizontal view.

It is important to recognise that medial orbital wall fracture results in the development of communication between the orbit and nasal cavity increases the risk of infection of the orbit tissues by nasal bacteria, which has the potential to spread into the cranium. These cases demonstrate that there is a risk of an associated ocular injury, so multidisciplinary management of these injuries by a surgeon and an ophthalmologist is recommended.

In summary, this clustering of six cases in 5 years suggests that this type of injury may be more common than the existing literature suggests. The combination of subtle signs and difficulties imaging to establish a diagnosis should be remembered by all those who treat facial injuries in children. Finally, such injuries are best treated in centres where multidisciplinary management can be undertaken.

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CHAPTER FOUR

INTRODUCTION

Tumour management in the Craniofacial region has been undertaken by a number of surgical specialties including Otolaryngology, Oral and maxillofacial surgery, Neurosurgery, General surgery, Ophthalmology and Plastic surgery, depending on tumour type, location and established local practice. Despite this there are children with tumours who present to a Craniofacial Unit where the surgical techniques employed by craniofacial surgeons together with the unit's established routine of long term multi-disciplinary management may be of great value in providing coordinated management and rehabilitation of children with particular tumours in this region.

In these seven papers there are examples of rare benign and malignant tumours, where the clinical descriptions warrant further publication, examples of management and also a long term outcome which highlights the problems of sequelae which may present to the surgeon even following successful eradication of the primary tumour by chemotherapy and radiotherapy.

CHAPTER FOUR - PAPERS

The first paper is a case report of a very rare congenital tumour in an infant. The paper describes the clinical features and treatment and compares this to the few existing cases in the literature.

The second paper is a case report of an odontogenic tumour which usually presents in teenagers but had only once been previously reported in such a young child. The clinical features are described in detail and the association of this tumour with cutaneous malignancy is critical requiring long term follow up of this case.

The third case is a clinical description of a curious finding in siblings having the same anomaly (a dermoid cyst) occurring in the same position on the nose. These are thought to be isolated events but these two cases in siblings are curious and raise the possibility that this family may have had an underlying predisposition to this anomaly.

The fourth paper concerns tumours arising from all three germ cell layers, the teratoma's. It considers the different clinical features and reports some of the challenges of management which not only relates to removing the tumour but includes treating the growth anomalies which affect facial development until skeletal maturity is reached.

The fifth paper is a clinical description of a rare tumour in a teenager which is reported to have a high incidence of metastatic disease, (usually occurring about five years later following tumour

excision). The treatment regime used for this patient included a technological innovation used in melanoma management – sentinel node biopsy to try to prevent this poor outcome by more accurately staging the tumour. Ten years later the individual is disease free indicating that at least in this case it appears to have been a useful approach.

The sixth paper is again a small series of an uncommon tumour usually treated by neurosurgeons. Access to the cranial base by a craniofacial surgeon can improve access for the neurosurgeon, with an improved chance to achieve complete surgical excision and improve long term outcome.

The seventh and final paper is a report of a man who underwent combined radiotherapy and oncological treatment for one of the commoner head and neck tumours of childhood, a rhabdomyosarcoma. This results in many treatment difficulties for the reconstructive surgeon which this paper highlights, but with persistence rehabilitation can be achieved, by a multidisciplinary team.

In summary these papers contribute to the clinical features of some rare tumours and describe management strategies for a few types and review long term outcome of some individual cases. Larger long term studies are needed to critically assess current treatment strategies for individual tumour types.

Congenital gingival granular cell tumour

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J R Soc Med 1996;89:53P-54P

SECTION OF PLASTIC SURGERY, 8 NOVEMBER 1994

Keywords: congenital, gingival granular cell tumour, neonate

Congenital gingival granular cell tumours are rare lesions which have only occasionally been reported in the UK. Clinical features are of a benign lesion which occurs almost exclusively in newborn, Caucasian females and the anterior maxilla is the commonest site. Treatment consists of local excision and is curative.

The terminology concerning this condition has been rather confused because of uncertainty regarding the histogenesis of these tumours and the similar histological appearance to adults granular cell myoblastoma occurring at other intraoral sites. The exact histogenesis of these tumours remains unresolved and they may be hamartomata.

We describe a new case occurring within the UK, which illustrates many of the common clinical features of the condition, with an accompanying literature review.

CASE HISTORY

A Caucasian female was referred a few hours after birth for urgent plastic surgical assessment with an intraoral swelling which was interfering with attempts at feeding. She had been born at term by a normal vaginal delivery following a normal pregnancy. No other abnormalities were found. It had been noted that she had a polypoid, congenital swelling $3 \times 3 \times 0.9$ cm which was superficially ulcerated arising from the anterior maxillary alveolus (Figure 1). The differential diagnosis included: odontogenic tumour, teratoma, neuroectodermal tumour and congenital granular cell tumour.

The initial management included intravenous fluids and nasogastric feeding. As oral feeding was clearly impossible for this child while this swelling remained, arrangements for early elective removal were made. The tumour was excised

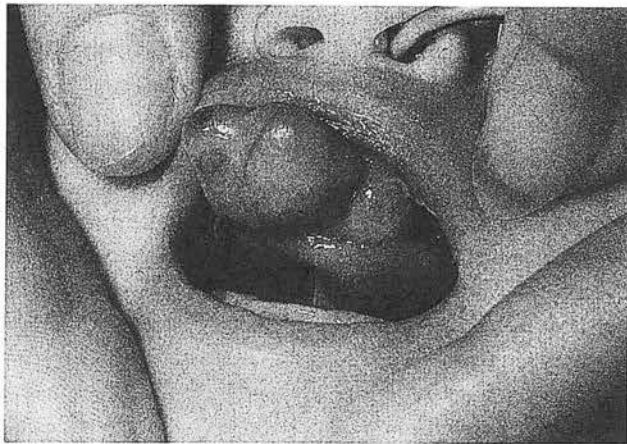


Figure 1 The preoperative appearance of the tumour showing it arising from the anterior maxillary alveolus (note the nasogastric tube for feeding)

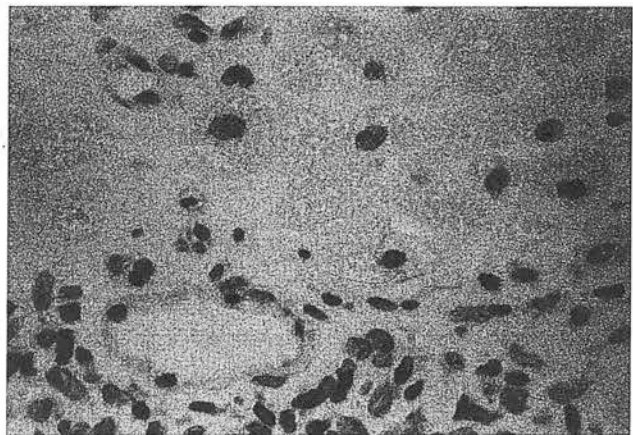


Figure 2 Section through the tumour ($\times 400$) stained with haematoxylin and eosin, demonstrating characteristic large round cells containing granular cytoplasm and a central nucleus

leaving the defect to heal by secondary intention under general anaesthesia, two days after birth. The patient was discharged home later the same day as surgery as oral feeding was readily established post-operatively.

Histopathology confirmed the diagnosis of a completely excised congenital gingival granular cell tumour (Figure 2). Immunohistochemical investigation confirmed the diagnosis.

Three months post-operatively the child is thriving with no evidence of recurrence.

DISCUSSION

Congenital gingival granular cell tumours have usually been sporadically presented as isolated case reports in British literature¹⁻⁴, since the first case was described in Germany in 1871⁵. The largest reported series comes from data collected throughout the USA over a period of 30 years, which reviews 21 of these lesions⁶.

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This case demonstrates several features which are characteristic of the condition. The child was female and Caucasian. The female to male ratio of this condition is 10:1⁷, although a male has been reported in the UK population². Where the racial origin of the child has been reported, only two cases worldwide occurred in non-Caucasians⁶. The mass occurred on the anterior maxillary alveolus, which has been reported as being affected twice as often as the mandible⁶. However, this lesion was unusually large at 3 × 3 × 0.9 cm; they are usually less than 1.5 cm diameter⁷. Local excision is curative for the condition with no reported recurrences in the literature, even when the excision has been incomplete⁶. It has been reported that the natural history of the condition is for the lesion to spontaneously diminish in size⁸, and that treatment should be conservative unless feeding was interfered with⁹ (as in this case).

The terminology of this condition has been confusing due to continuing uncertainty regarding the histogenesis of the lesion. Both mesenchymal and odontogenic origins have been suggested⁷, and the clinical behaviour has led to the suggestion that they may be considered hamartomata rather than neoplasms⁹. The term 'epulis' has been used, but this simply means swelling on the gingiva and lesions with quite different pathologies have been grouped together. This accurate diagnosis has been further complicated by the similar histological appearance to the oral granular cell 'myoblastoma' which occurs in adults at a number of intraoral sites, most commonly on the tongue. However, histological differences are noted in that the covering epithelial hyperplasia so often noted in adult granular cell tumour is absent in the congenital tumour⁷.

Immunohistochemical staining can be of use in distinguishing the two conditions although there is some

variation in the results obtained. In the noncongenital granular cell tumours there are usually positive responses to antigens for S100, and variable responses to myoglobin, myosin, actin, desmin, α -1-antitrypsin and muramidase. Congenital gingival granular cell tumour may stain positive for actin and myosin but not S100 as in our case, but other authors have found them negative for all markers¹⁰.

In summary a case of congenital gingival granular cell tumour is reported which on reviewing the literature, exhibits many of the described clinical features but in addition is an unusually large example of this rare lesion.

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CASE REPORT

Odontogenic keratocyst in a 5-year-old child: a rare cause of maxillary swelling in children

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KEYWORDS

Maxillary swelling;

Paediatric;

Odontogenic keratocyst

Summary Odontogenic keratocysts in children are uncommon. They are cysts of the jaws that have a tendency for recurrence and are usually seen in adults. We report an exceptionally rare case in a young child and discuss its management.

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A 5-year-old-boy was referred to the Australian Craniofacial Unit, Adelaide, Australia, with a 6-month history of a slowly enlarging mass on the left side of his face. The mass was painless, there was no history of nasal discharge, and there was no visual disturbance. Examination revealed a swelling of the left cheek (Fig. 1). Intraorally, there was swelling of the buccal and palatal aspects of the upper left quadrant. There were numerous carious deciduous teeth (Fig. 2).

Computed tomography scans showed an expansile mass within the left maxilla, with associated displacement of the teeth in that quadrant (Figs. 3 and 4).

The differential diagnosis included benign periodontal or dentigerous cyst, odontogenic keratocyst or a malignancy such as rhabdomyosarcoma or ameloblastoma. A biopsy was taken of the lesion in order to establish a diagnosis.

The boy was given a general anaesthetic and via a Caldwell Luc approach, the lesion was found to be cystic. The cyst was enucleated and the cavity curetted. Two displaced teeth, attached to the cyst wall, were removed.

Histological analysis of the cyst lining confirmed the lesion to be an odontogenic keratocyst. The histological features of the cyst lining, which are characteristic of an odontogenic keratocyst,¹ are shown in Figs. 5 and 6.

Discussion

Odontogenic keratocysts are thought to be developmental in origin, arising from embryonal dental tissues.² They usually present as a slowly enlarging, painless swelling,

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Figure 1 'Worms eye' view showing unilateral swelling of the left cheek.



Figure 2 Intra-oral examination showing carious teeth and buccal swelling.

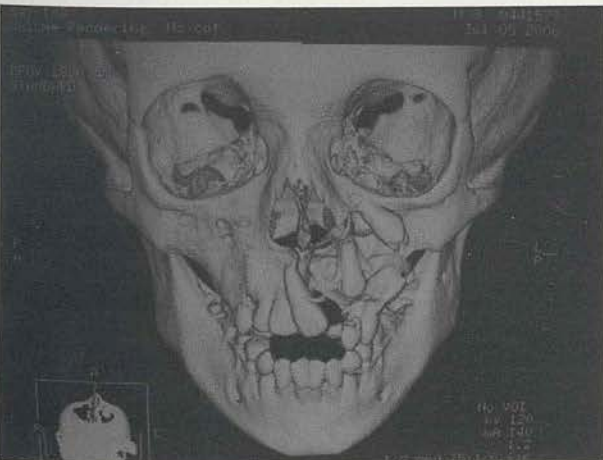


Figure 3 Defect and displaced teeth.



Figure 4 The extent of the lesion.

typically in the second or third decades of life, and are very rare in children. Less commonly, there may be spontaneous drainage of the cyst fluid, pain, cellulitis, trismus or nasal discharge.² Radiographs typically show a unilocular well circumscribed, radiolucent lesion often associated with displacement of a tooth. A number of treatment options are available after open biopsy to confirm the diagnosis of odontogenic keratocyst. The more conservative approach to odontogenic keratocysts is enucleation and curettage. The more aggressive treatment regimens include cryotherapy, Carnoy's tissue fixative solution or, rarely, maxillectomy

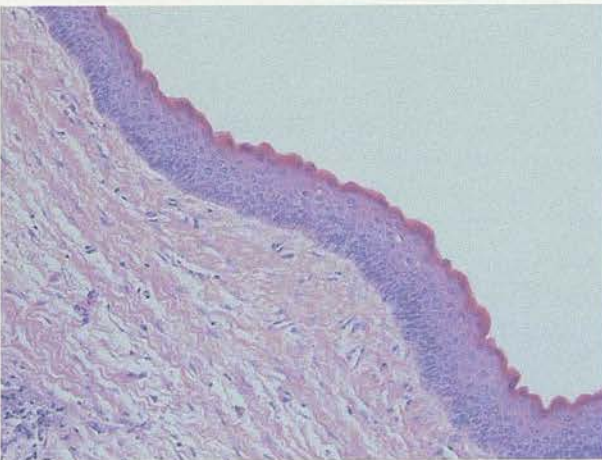


Figure 5 Histological section. H&E, 200x Thin stratified squamous epithelium, which overlies fibrous connective tissue. Prominent cuboidal to columnar basal cell layer, no rete ridges and an undulating surface layer of parakeratin.

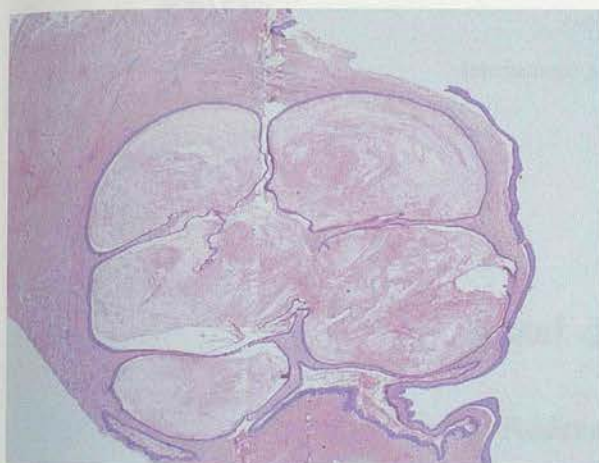


Figure 6 Histological section. H&E, 20x The cyst wall contains a number of 'daughter cysts', also lined by squamous epithelium.

or mandibulectomy. Odontogenic keratocysts are well known for their aggressive behaviour and propensity for recurrence. Recurrence rates between 2.5 and 62.5% have been reported.³ A number of theories about the high recurrence rate have been proposed, including incomplete removal of the original lesion, remnants of the dental lamina within the jaws and the presence of 'daughter' or 'satellite' cysts within the epithelial cyst wall.² The use of Carnoy's solution or cryotherapy is thought to eliminate possible satellite cells.

In children, the mean age of presentation of odontogenic keratocysts is 3.4 years (range 8–18 years), and occurs slightly more commonly in the mandible compared with the maxilla. There is a slight male predominance.² Odontogenic keratocysts can arise sporadically or may be associated with nevoid basal cell carcinoma syndrome (Gorlin's syndrome). Gorlin's syndrome is inherited as autosomal dominant, and

is associated with a number of anomalies, including multiple basal cell carcinomata, odontogenic keratocysts, palmar pits, skeletal abnormalities, the most frequent of which is bifid ribs but also includes vertebral abnormalities such as kyphoscoliosis and spina bifida. Intracranial calcification may also occur, and this usually manifests as calcification of the falx cerebri, the sella turcica, tentorium cerebella, or both.⁴

Odontogenic keratocysts are an uncommon cause of facial swelling in a child. They are rare in children and exceptionally rare in children under the age of 10 years.⁵ When they are discovered in this young age group, they may be associated with nevoid basal cell carcinoma syndrome, and may be the first clinical sign of the syndrome.⁶ As plastic surgeons, it is important that odontogenic keratocysts should be included in the differential diagnosis, as it has consequences for their long-term management. These patients therefore require long-term follow up for recurrence of the keratocyst and also for the subsequent development of the signs and symptoms of nevoid basal cell carcinoma syndrome.

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Case report
Nasal dermoid cysts in siblings

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Abstract

Nasal dermoid cysts are congenital malformations which result from anomalous embryological development. Two cases occurring in siblings are presented. There have been several previous reports that the condition may be familial but in this report the initial suspicion of the milder anomaly in the younger child was raised primarily because of his older brother's history. This further report suggests that the incidence of familial occurrence of this anomaly may be greater than current literature suggests, and the possibility that other family members may be affected should be remembered by clinicians treating patients with this condition. © 1998 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Dermoid cyst; Congenital malformation; Siblings

1. Introduction

Nasal dermoid cysts are the result of anomalous embryological developments and occur in the midline of the nose and walls contain skin adnexae [1–5]. Congenital dermoid cysts of the nose are rare, but well recognised malformations which have been described regularly since the first report in 1817 [5]. Dermoid cysts in the nose is an unusual site with an incidence of just 3% of all dermoids [6].

The anomaly can occur as an isolated finding or in conjunction with other anomalies in up to 41% of cases [7]. The presentation is commonly due to nasal swelling or the observation of a midline sinus tract, although large cysts can produce nasal obstruction [6]. However, there has been a wide range in the age of presentation which has been reported to range from 3 months to 59 years, with a median age of 12 years [6]. The differential diagnosis includes epidermal cyst, abscess, mucocele, frontal bone osteomyelitis, sebaceous cyst and nasal glioma [2,5].

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Familial occurrence of this condition has previously been reported on occasion [5,8–13]. The following report illustrates how the anomaly was recognised in a younger sibling only after his elder brother had undergone treatment for a nasal dermoid.

2. Case report

A 2 year old boy was referred for plastic surgery opinion with a 4 month history of swelling of the bridge of the nose and intermittent discharge from a sinus 1 cm below the swelling (Fig. 1). The diagnosis of midline nasal dermoid cyst with an accompanying sinus was made. Radiological examination confirmed the presence of a

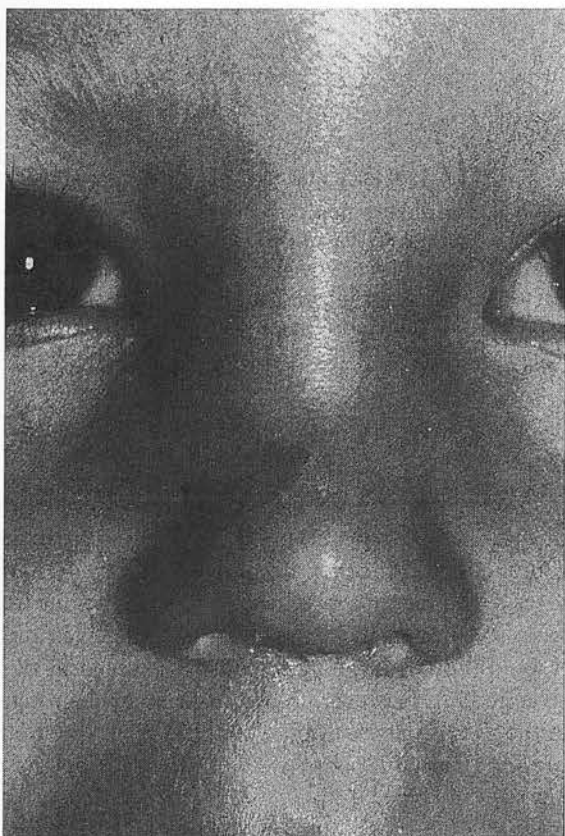


Fig. 1. Photograph of case one, aged 2 years, demonstrating the sinus on his nose.

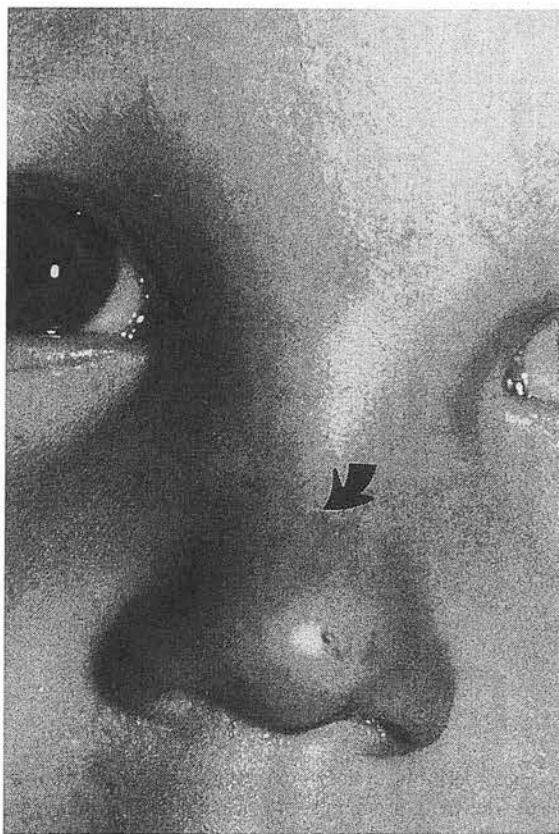


Fig. 2. Photograph of the younger sibling, case two, 1 year old demonstrating a clinically less obvious sinus (arrowed).

space occupying lesion without intracranial extension, the findings in keeping with a diagnosis of nasal dermoid cyst.

Under general anaesthesia, excision of the midline sinus and extension of the incision in the midline was made to allow access. The sinus was traced superiorly and extended below the nasal bones. The cyst was removed after the nasal bones had been elevated, attached to a hinge of periosteum, and the resulting cavity curetted. The nasal bones were repositioned and the wound closed in layers. The patient made an unremarkable recovery and was discharged the following day.

On review 6 weeks later, the area was healing with a satisfactory early result. The mother also brought his younger sibling aged 1 year in whom she had noted had a similar nasal sinus (Fig. 2),

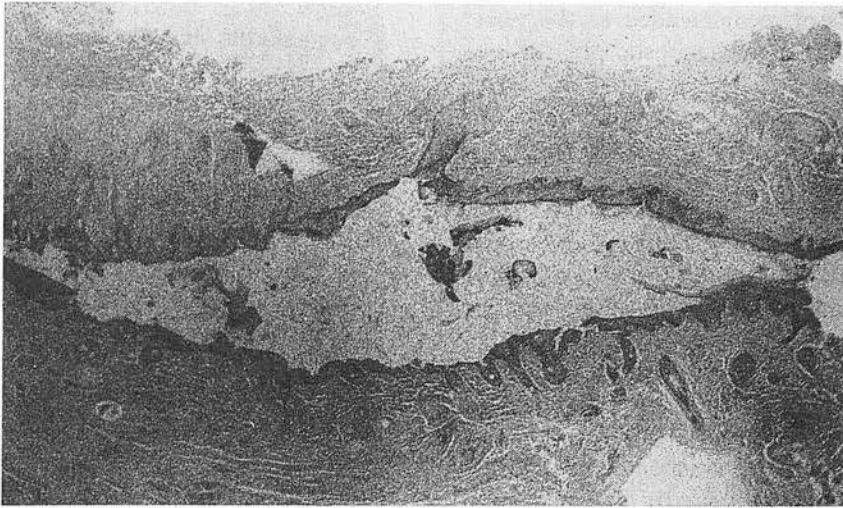


Fig. 3. Histological examination of the cyst removed from case two, demonstrating the cyst wall lined by keratinizing stratified squamous epithelium and adnexal structures thereby confirming the diagnosis of a dermoid cyst. Stain H and E. Original magnification $\times 40$.

and wondered whether surgical exploration was required. Curiously, she recalled that her grandmother and great-grandfather had similar nasal pits but had never undergone surgery prior to their demise.

The younger son also underwent radiological examination which confirmed the presence of a mass in keeping with a diagnosis of a dermoid cyst. Surgical removal was performed similar to that undertaken on his brother, and a smaller cyst was removed. This too was confirmed as a nasal dermoid on histological examination (Fig. 3). He also made a remarkable recovery post-operatively. He was reviewed in the clinic with his elder brother 6 weeks post-operatively, his elder sibling was by then, 8 months post-operatively and both were symptomless. The early cosmetic result is shown (Figs. 4 and 5).

3. Discussion

Extensive review of the literature revealed several previous reports of this condition occurring in family members [5,8–13]. However, these previous reports span over 40 years and have appeared in a variety of speciality journals, so the full

extent of familial nasal dermoids may not have been readily apparent.

These previous reports included both affected siblings [5,9–11,13], as well as parents and their children [5,8,10–13]. In this family, the history of nasal pits in both grandmother and great-grandfather raises the possibility that there was a family predisposition to this condition. It is particularly important that it was only because of the treatment of the elder brother and the recognition of a nasal pit by the mother, that surgical referral of the younger sibling occurred at all.

The association of nasal sinuses with dermoid cysts of the nose, as demonstrated in our cases, is well recognised [1,4,5,14,15]. The position of a sinus from a nasal dermoid is variable and can occur anywhere between the glabella and the base of the columella [4], although the lower third of the bridge of the nose has been reported to be the most common site by several authors [1,2,5].

The management is surgical excision but careful preoperative investigation is essential since cysts may extend to involve the septum, cribriform plate, or the dura. MRI scanning [16], CT scanning [14], and conventional radiographs (using antero-posterior, lateral and Water's views) [6], have all been advocated to identify any cyst exten-

sion. MRI has been recommended as the treatment of choice, although diagnostic pitfalls have been reported [17].

Treatment in these cases consisted of excision of sinus in continuity with the dermoid cyst via a midline incision on the nose. Other approaches via the forehead have been described [2,15], and a combined craniofacial approach using a bicoronal flap as well as a nasal excision to remove a sinus has been recommended for extensive lesions [4,14,18].

Reported complications in addition to the inevitable facial scarring have been reported and include saddle nose deformity [19], infection [15] and recurrence if removal is incomplete [15]. Destruction of any residual lining by electrocoagulation has been advocated to prevent recurrence [6], although the development of craniofacial techniques where access is difficult should reduce the need for this.

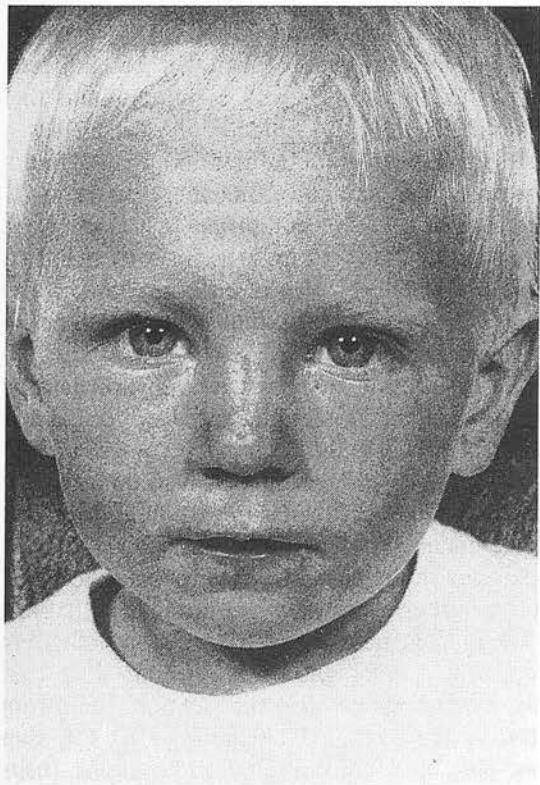


Fig. 4. Photograph of case one 8 months following surgery.



Fig. 5. Photograph of case two 6 weeks following surgery.

4. Conclusion

In conclusion, nasal dermoid cysts have been demonstrated in siblings who had a family history of nasal pits. In this case, the recognition of an existing nasal pit in the younger sibling was an essential guide to the existence of the anomaly. The surgical management of the condition has been reviewed.

This further example of familial nasal dermoid cysts in addition to the existing reports highlights, to clinicians treating this anomaly, the possibility that other family members of an affected individual may also have the condition.

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Teratomas of the head and neck region

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SUMMARY. Background: Teratomas are exceptionally rare malformations in the head and neck region. They are mostly benign but as a direct result of their rarity, most clinician's experience of these tumours is very limited, and consequently most of the associated literature consists of single case reports. In this paper, however, all the cases managed by a major Craniofacial Unit (the Australian Craniofacial Unit) were reviewed to attempt to identify common problems encountered in their management. Material and methods: All cases managed by the Australian Craniofacial Unit over the last 25 years were reviewed. In total a series of nine cases was identified, but two were seen and operated on in overseas centres and the data in these cases were incomplete, and have been excluded from the study. Case note, radiology and pathology review was undertaken to collect data. Results: In total a series of seven cases was identified as suitable for inclusion in this study. Six of these have had a minimum of 9 years follow-up, three having completed growth. Conclusion: The initial and subsequent management demonstrates that these tumours when benign can be successfully removed, but depending on the affected site may require continued multidisciplinary management until growth has finished. © 2003 European Association for Cranio-Maxillofacial Surgery.

Keywords: Teratoma; Craniofacial

INTRODUCTION

Teratomas are exceptionally rare malformations in the head and neck region. This has resulted in papers pertaining to the management of these conditions being mostly limited to single case reports. In this paper, however, all cases managed by the Australian Craniofacial Unit (ACFU) over the last 25 years were reviewed to attempt to identify common problems encountered in their management. In total a series of nine cases was identified but two were seen and operated on in overseas centres and the data were incomplete, leaving seven cases. Six of these have had a minimum of 9 years follow-up, three having completed growth. Their initial and subsequent management and their long-term outcomes have been summarized in Table 1 and shall be described in detail.

CASE REPORTS

Case 1

A Caucasian female was born by vaginal delivery with a soft blue swelling visible in the neck (Fig. 1). She had inability to swallow, airway obstruction and developed a bilateral pneumothorax, requiring intubation. She was referred to the ACFU for multidisciplinary management. A CT scan demonstrated a tumour extending from the pharyngeal wall to the base of the skull and laterally into the left middle ear canal. Angiography was attempted but unsuccessful.

At 2 weeks of age she underwent subtotal removal of the tumour in a combined procedure with an ENT surgeon. Histology confirmed the diagnosis of benign teratoma. Her remaining problems included the remaining tumour in the middle ear canal, along with weakness of the marginal mandibular branch of the facial nerve and for the contour deformity of the left side of her face. The facial nerve palsy settled over the next 2 years.

At 4 years of age a recurrent tumour was removed from the left nasopharynx and soft palate via a mandibular osteotomy on the left side. The defect was repaired using a pharyngeal flap. Post-operatively, there was thought to be a palsy of the IX cranial nerve. This manifested in swallowing and speech difficulties. These improved with speech therapy and resolved completely. However, she was left with a significant contour defect in her neck (Fig. 2).

Six years later when aged 10 years a lateral arm free tissue transfer was undertaken to correct the contour deformity of the left side of the face and neck. Additionally, throughout childhood she has had long-term Eustachian tube dysfunction, which required the insertion of grommets on two separate occasions.

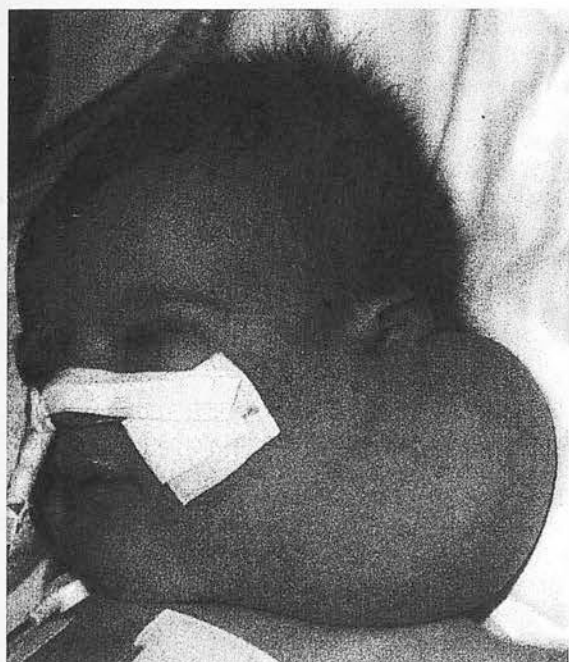
In total there were three further operations following tumour removal, and in addition she had regular long-term speech therapy.

Case 2

A Caucasian female was born by vaginal delivery, with an older non-identical twin. She was noted to have a swelling over the right temporal area and extensive intraoral swelling extending to involve the

Table 1 – Teratomata treated by the Australian Craniofacial Unit 1975–2000

	Age at referral	Current age	Sex	Country of origin	Site	Number of operations	Previous operations
1	Birth	17	f	Australia	Parapharyngeal space	5	None
2	Birth	18	f	Australia	Infratemporal fossa	4 ^a	None
3	2 months	Deceased	m	Hong Kong	Intracerebral (anterior cranial fossa)	3	None
4	2 years	9	f	Malaysia	Left parapharyngeal space	2	1
5	Birth	14	f	Australia	Left temporal lobe, parapharyngeal space	4 ^b	None
6	Birth	11	m	Australia	Infratemporal fossa	3 ^b	None
7	3 years	22	m	Malaysia	Right pterygoid fossa	2 ^b	2

^aFurther surgery offered and declined.^bFurther surgery planned.**Fig. 1** – Case no. 1. Swelling in the left neck.

soft palate which prevented feeding (Fig. 3). She had respiratory distress and was intubated and was referred to the ACFU for further management. CT scan demonstrated a tumour within the right infratemporal fossa. Angiography was attempted but unsuccessful.

Four weeks post-partum she underwent elective tracheostomy, prior to definitive excision 1 week later. It had been planned to leave this until she was 3 months old, but because the tumour was increasing in size, earlier intervention was undertaken. Access for tumour removal was via a bicoronal scalp flap, osteotomy of the zygomatic bone and maxillary split. The tumour was removed completely and the malar bone fixed back into position. The temporalis muscle was split to fill the defect between the base of the skull and the oropharynx and the remainder to fill the temporal fossa. Post-operatively feeding was slow due to poor palatal function, the tracheostomy was closed 1 month after surgery, and feeding improved after this. Six months later palate repair was undertaken. This was done using

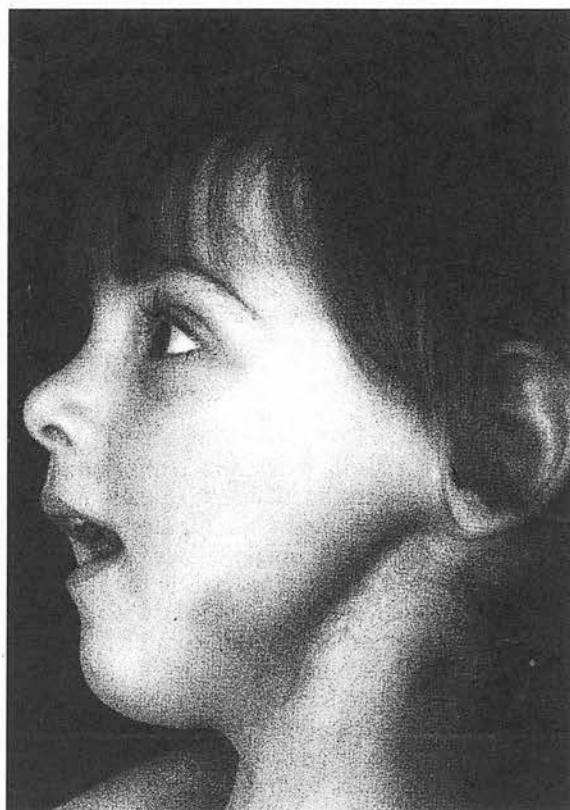
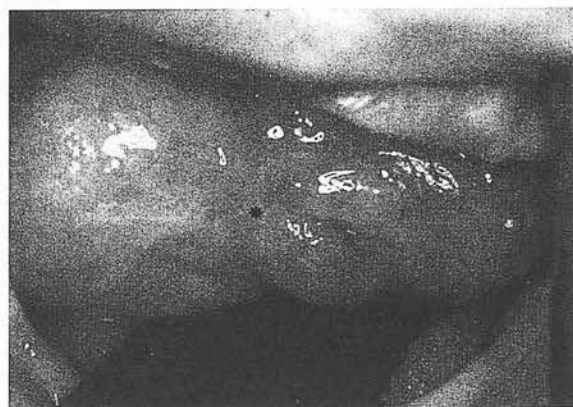
**Fig. 2** – Case no. 1. Marked contour deformity with a central scar on the left side of the lower face.**Fig. 3** – Case no. 2. Tumour mass in the palate, marked with an asterisk.



Fig. 4 - Case no. 2. Facial asymmetry due to temporal hollowing on the right side.

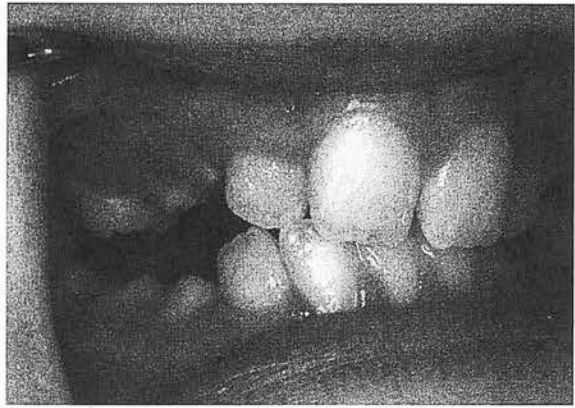


Fig. 5 - Case no. 2. Open bite on the right side, age 9 years.

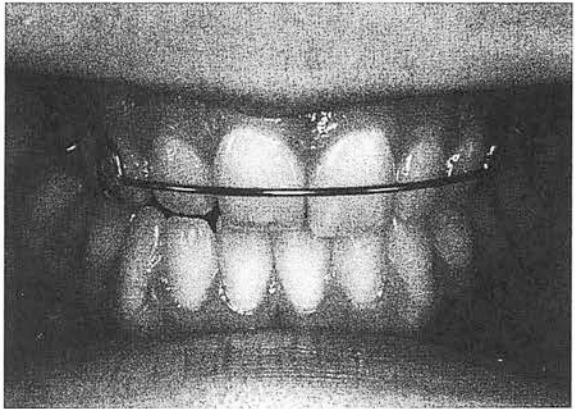


Fig. 6 - Case no. 2. Occlusion following orthodontic treatment, age 15 years.

a modified Veau-Wardill-Kilner pushback, which was complicated by distortion of the right palatal shelf due to the temporalis flap reconstruction, which had produced a shorter palatal shelf than on the left side. Within 3 months she was noted to have similar speech to her twin. By the age of 6 years speech was markedly hypernasal. Nasendoscopy was undertaken and showed little movement on the right side. Shortly afterwards she underwent a pharyngoplasty with thinning of the temporalis flap. This produced a dramatic reduction in the hypernasality.

By the age of 8 years she was noted to have mild facial asymmetry with a right temporal hollowing and her chin pointing to the right (Fig. 4). She also developed a right open bite (Fig. 5) for which she was treated orthodontically. By the age of 15 years she had an acceptable occlusal relationship with no cant (Fig. 6). However, she still had residual temporal hollowing (Fig. 7), and was offered further intervention in the form of fat injections but declined.

In total to reconstruct the consequences of tumour removal she has had two further operations to improve palatal function, speech therapy and orthodontics.

Case 3

A Chinese male was born by Caesarian section at 37 weeks gestation. He had marked enlargement of his

cranium, swelling in the central face in the left supraorbital region producing asymmetry, and a complete left sided cleft lip, alveolus and palate (Fig. 8). The baby had no difficulty with breathing and feeding.

CT scan demonstrated a large intracerebral teratoma occupying both sides of the anterior cranial fossa. There was no normal cerebral tissue in the supratentorial region, although the infratentorial tissue looked normal. There were two extracranial tumour extensions in the left orbit. An angiogram was undertaken and demonstrated that the ophthalmic vessels were tortuous and were the main blood supply to the tumour. He was referred to the ACFU for further management.

He underwent multidisciplinary review and despite his anomalies, he was an alert baby breathing without difficulty. Three months post-partum he underwent removal of the intracranial tumour, via an anterior craniotomy. This was encapsulated (Fig. 9). However, the mass in the temporal region was noted to arise within the temporal lobe, was also excised and sent for histological examination, leaving the surrounding temporal lobe tissue. The dura was closed and the calvarium plicated to reduce the extensive

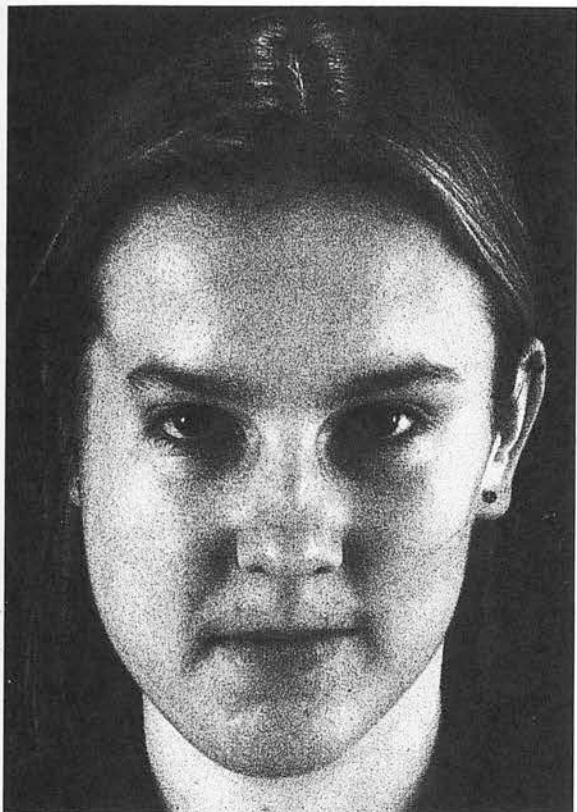


Fig. 7 – Case no. 2. Remaining contour defect of the right temporal region.



Fig. 8 – Case no. 3. Infant with marked swelling over the left supraorbital and nasal regions. Note the associated cleft.

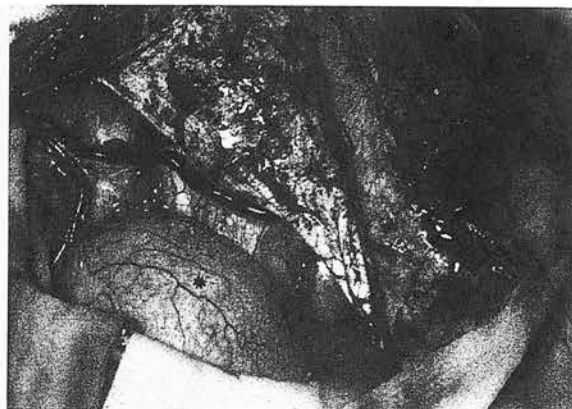


Fig. 9 – Case no. 3. Teratoma intraoperatively, which is well demarcated, surrounded by dura, within the anterior cranial fossa and marked with an asterisk.

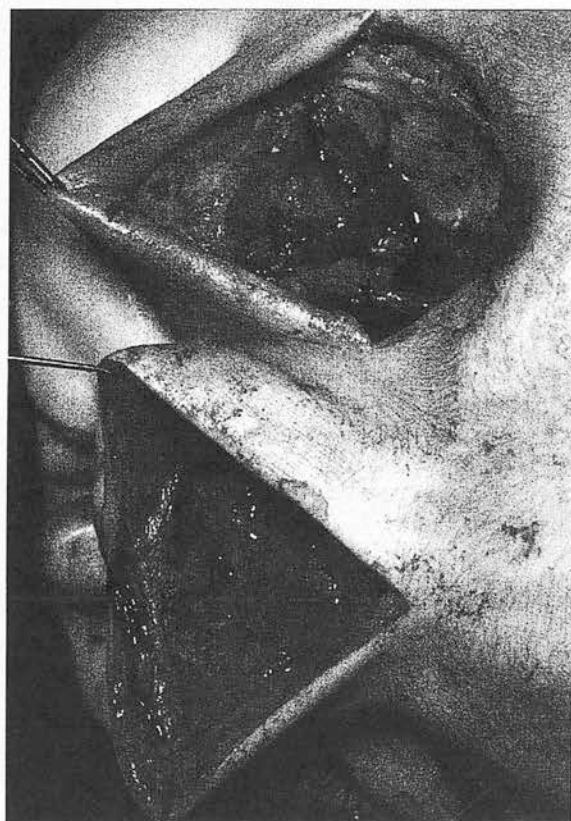


Fig. 10 – Case no. 3. Approach to the extracranial tumour extensions in the midline of the nose and on the left forehead.

dead space. Histological examination reported the specimens to contain a benign teratoma. Post-operative recovery was uneventful. Two weeks later the extracranial tumour extensions were excised from the left supraorbital region (Fig. 10) and the cleft lip and palate repair was undertaken. Histological examination revealed a benign tumour, with a small focus of infantile embryonal carcinoma, which was reported to have been completely excised.

He underwent a minor revision to improve the nasal appearance 2 months later and transferred



Fig. 11 – Case no. 3. Appearance after the third operation before returning to Hong Kong.



Fig. 12 – Case no. 3. Recurrent tumour in the orbital region 3 months after returning to Hong Kong.

home to Hong Kong (Fig. 11). Three months later a growth developed rapidly in the left supraorbital region (Fig. 12), and he was transferred for further assessment at the ACFU.

There was massive growth in the left orbital region, and no evidence of metastatic spread on imaging studies. The CT scan did reveal a massive growth with erosion of the maxilla, zygoma and nasal region (Fig. 13). Biopsy was undertaken and confirmed the presence of infantile embryonal carcinoma. Due to the size of the tumour further resection was not attempted. No further intervention was attempted after discussion with the parents, and the child was discharged home and died a few weeks later.

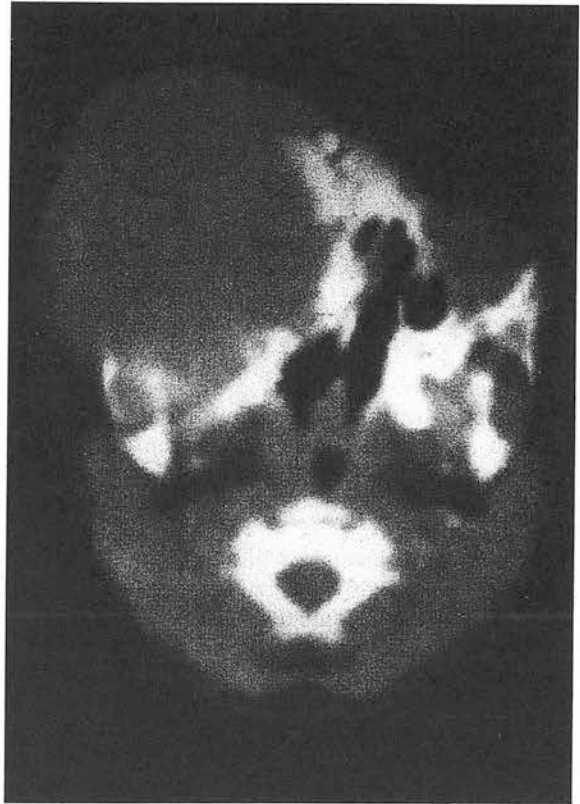


Fig. 13 – Case no. 3. CT scan demonstrating massive recurrent tumour in the orbital region with major destruction of the facial skeleton.

Case 4

A Chinese female was born by vaginal delivery at term. She was noted to have marked deformity of her face with swelling over the left side of her lower face and neck. Intraoral examination revealed a cleft of the soft palate. She required hospitalization for airway management and nasogastric feeding. Examination under anaesthesia and biopsy to establish the diagnosis were undertaken prior to her referral to an overseas ACFU clinic in Malaysia.

After delay due to parental anxiety regarding travel, she was seen 2 years later in the ACFU and underwent multidisciplinary evaluation and MRI scan. This demonstrated the tumour extending from

the lateral pharynx into the left temporal region. The left-sided facial swelling had been increasing in size (Fig. 14). The tumour was removed via bicoronal and submandibular incisions. The zygomatic arch was osteotomised to allow access to the tumour. Intraoperatively the pharyngeal wall was breached but immediately repaired. The tumour was of variable consistency partly hard, partly soft and was removed completely although the margin was minimal where the tumour was peeled off around the facial nerve.

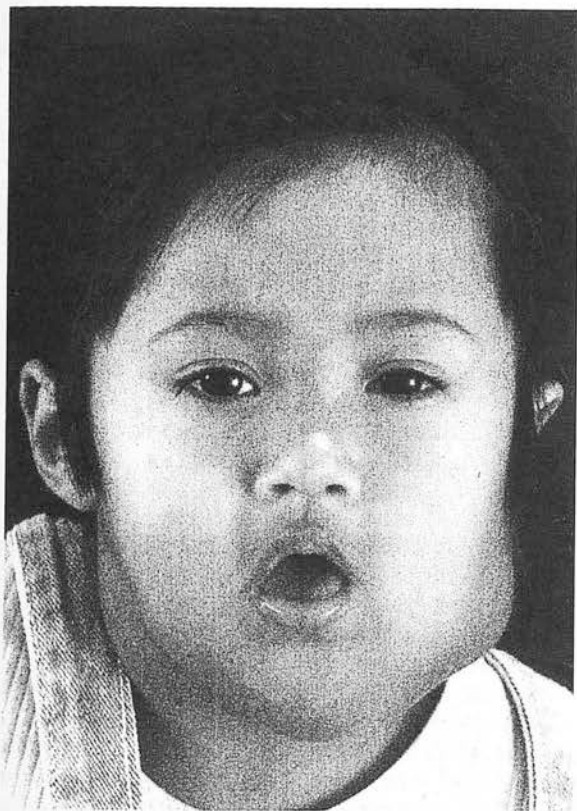


Fig. 14 – Case no. 4. Marked swelling over the left angle of the mandible.

Post-operative recovery was unremarkable and 3 weeks later she underwent cleft palate repair and insertion of grommets. She was discharged home to Malaysia 2 weeks later and remains under yearly review. Her speech is satisfactory and she has no other complications.

She has had just the one operation following tumour removal although she is likely to require orthodontic treatment in the future.

Case 5

A Caucasian female was born by vaginal delivery at 37 weeks gestation. She was noted to have a swelling in the left temporal region, which was identified to be a 5cm cystic mass in the left temporal lobe by ultrasound examination. She was referred to the ACFU where she underwent multidisciplinary assessment along with MRI and CT scans. These identified a defect in the middle cranial fossa, and also a lesion on the left cervical region down to the clavicle. It was thought that this represented a teratoma with intracranial extension.

She underwent excision of the temporal tumour by a neurosurgeon 1-week post-partum. Post-operatively she developed a left III nerve palsy which required a tarsorrhaphy. The cervical component became clinically more obvious after the intracranial component had been removed and swelling on the left side of the neck was seen. When she was 3 months old

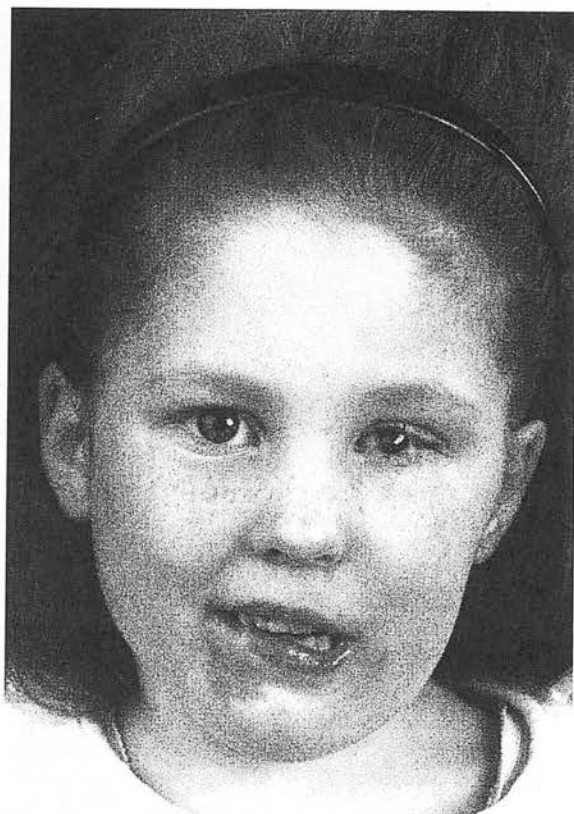


Fig. 15 – Case no. 5. Facial asymmetry, seventh nerve palsy affecting the left side, and skin excoriation below the right lower vermillion.

the remaining tumour was resected through a submandibular incision. Two apparently separate encapsulated masses were identified and removed intact. No direct communication with the base of the skull was identified. Despite the suggestion by the pre-operative scans that the two tumours were connected, this could not be confirmed macroscopically or by subsequent histological examination.

Post-operatively she developed a partial left sided VII nerve palsy. She required considerable speech therapy for receptive and expressive delay but by age five had made significant improvement. At age 5 years the tarsorrhaphy was undone and a gold weight inserted by the ophthalmology team. There also was saliva dribbling from her mouth causing skin excoriation (Fig. 15). The recurrent excoriation of the perioral skin was managed by a dermatologist with barrier cream. Diversion of the submandibular ducts was contemplated, but was abandoned as the problem resolved spontaneously.

At age 10 years she required grommets for recurrent middle ear infection on the left side, related to poor Eustachian tube function. When reviewed at age 14 years she was noted to have persistent facial asymmetry due mainly to an asymmetrical mandible.

She has had two further operations after the neurosurgical tumour removal was undertaken, but in addition has also had prolonged speech therapy

and regular review throughout childhood by an ophthalmologist.

Case 6

A Caucasian male was born by Caesarian section at 37 weeks of gestation. He had unilateral facial swelling of the left cheek (Fig. 16). There were no airway or feeding difficulties initially but the child also had a moderately sized ventricular septal defect. After 2 weeks he required a nasopharyngeal airway tubing and was referred to the ACFU for further management.

A CT scan demonstrated a mixed density mass within the left infratemporal fossa, which was expanding laterally pushing the zygomatic arch (Fig. 17). The CT scan appearances were suggestive of teratoma but rhabdomyosarcoma could not be excluded, so biopsy was undertaken and the diagnosis confirmed. The decision was made to delay definitive surgery until the patient was 3 months of age when the tumour was excised via a bicoronal flap, and a zygomatic osteotomy, in conjunction with an intraoral incision. The defect was repaired with a temporalis muscle flap. Post-operatively he developed recurrent ear infections requiring grommet insertion twice and was left with a residual deformity of the left infratemporal fossa. This was accentuated by mandibular asymmetry resulting from ipsilateral abnormal development of the TMJ. He had no difficulties with feeding but had articulation problems with speech. Palatal reconstruction was undertaken at age

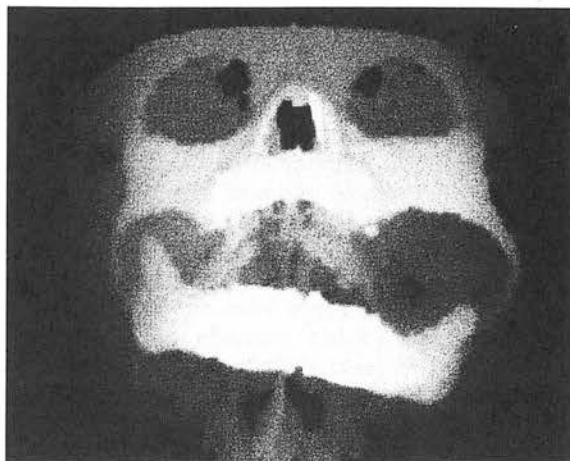


Fig. 17 – Case no. 6. Three-dimensional CT scan demonstrating the bowing of the malar arch on the affected side.

3 years using a modified Veau-Wardill-Kilner push-back, but he has persistent velopharyngeal incompetence and remains under the care of the speech therapists.

He is currently undergoing orthodontic treatment and a mandibular osteotomy is planned to correct the mandibular asymmetry. He will also be offered dermofat grafts to improve the contour of the temporal region.

He has had three further operations to improve palatal and ear function and has undergone extensive speech therapy. Two further operations are planned to improve facial cosmesis and masticatory function.

Case 7

A Chinese male born by Caesarian section at 37 weeks gestation presented with airway compromise and a mass affecting the right side of his face and right proptosis. After 3 weeks a tracheostomy was undertaken along with de-bulking of the tumour via an intraoral approach. He was hospitalized for 3 months. A further 3 months later he had recurrent tumour and further attempt at tumour removal via a Weber-Ferguson approach. Post-operatively no complications are recorded.

He presented 2 years later with a facial abscess at the site of tumour removal. A CT scan revealed further recurrent tumour extending from the right pterygoid fossa up to the level of the optic nerve. He was then referred to the ACFU for further management.

Multidisciplinary assessment found that he had right orbital dystopia and facial asymmetry with little vision in his right eye. The tumour was removed via a bicoronal scalp flap, with a right malar osteotomy (Fig. 18). Post-operatively, he developed an ophthalmoplegia but this resolved spontaneously over 2 weeks. He later developed recurrent infection along the lower right eyelid, this improved with antibiotics, but was finally treated by ophthalmic surgery by



Fig. 16 – Case no. 6. Left sided infratemporal swelling.

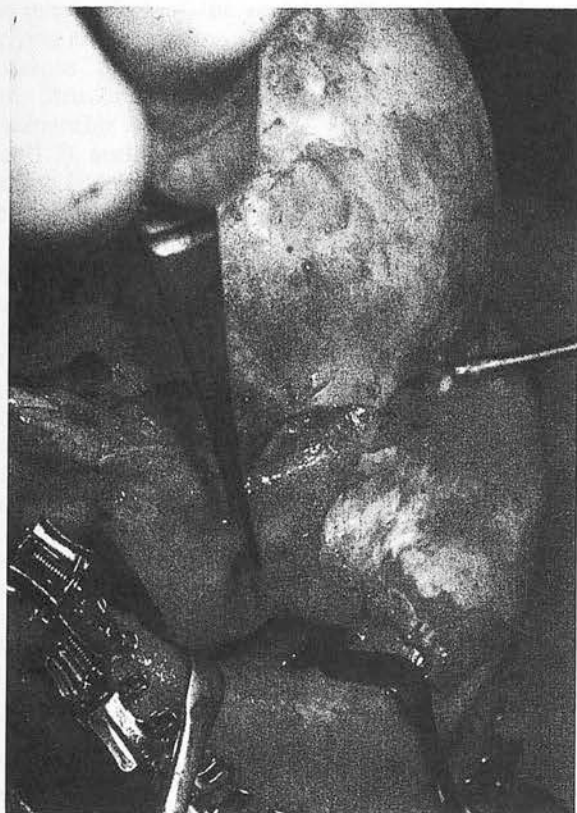


Fig. 18 – Case no. 7. Bicoronal flap and malar osteotomy allowing access to the tumour.

correction of an ectropion after 6 months. He has had no further problems with infection.

With growth he developed a cross bite on his right side, which is currently being treated by an orthodontist with a view to subsequently undergoing bi-maxillary surgery and genioplasty.

So although no further surgery following tumour removal has been undertaken as yet it is planned to improve masticatory function and facial cosmesis.

DISCUSSION

Teratomas have been reported to have an incidence of 1:4000 live births, but only 2% occur in the head and neck region (*Weaver et al., 1976; Holt et al., 1979*). As a direct result of their rarity most clinician's experience of these tumours is very limited, and consequently most of the associated literature consists of single case reports (*Montoya et al., 1980; Sadove et al., 1991; Spinelli et al., 1993; Hansen et al., 1995; Lanzio et al., 1998*). Even so-called multicentre studies have sometimes identified only a few cases (*Fearon et al., 1993*).

Teratomas are tumours containing components of all three embryological germ layers, unlike dermoids which contain tissue from two germ layers only (*Holt et al., 1979*). They are said to be benign, in 90% (*Fearon et al., 1993*), but malignant change although extremely rare in the head and neck region, has been

described (*Griffith, 1976; Holt et al., 1979*). This series demonstrates that a variety of sites in the head and neck region is possible (including intracranial and extracranial and occasionally both sites).

The patients in this series were referred both within the drainage area of the unit and from overseas but with the small number of cases no geographic or ethnic pattern could be discerned as a risk factor. Similarly, there were no apparent sex differences in the incidence.

Three of the cases required delivery by Caesarian section, but obstetric history was otherwise unremarkable. None of these cases had a diagnosis made pre-partum, and that may reflect obstetric practice at that time. Now that ultrasound scanning is more widely undertaken, it is possible that teratomas as well as other major craniofacial anomalies will be detected pre-natally in the future (*Wong et al., 2001*). An understanding of the natural history of this condition is essential for clinicians to enable informed parental choice regarding a possible termination of pregnancy. It may be a reflection of this that we have had no further cases referred in the last 9 years, and none from within Australia in the last 12 years. The presence of a co-existing cardiac anomaly was significant in case no. 6 since this resulted in the delay of the surgery until the child was 3 months old.

The investigation has been by three-dimensional CT scans with expert radiological interpretation. In some cases this has been supplemented by MRI scans and angiography. It is notable that in this series three cases had failed attempts at angiography.

It is significant that the tumours were benign apart from case 3 where within the recurrent tumour a small focus of malignant change was identified. This case was also the only one which subsequently resulted in a lethal outcome. This is in keeping with a previous report that only few infants with malignant head and neck teratomas survive (*Fearon et al., 1993*). However, it has been reported that the possibility of malignant change within a teratoma can be predicted by a raised serum alfa fetoprotein (*Takeuchi et al., 1979*). Interestingly, three of the other cases had undergone this examination as part of their investigations and it was normal in all of these cases.

The differential diagnosis of the masses noted in those patients includes: Encephalocele, heterotopic cerebral tissue, Neurofibromatosis, Klippel-Trenaunay-Weber syndrome, Proteus syndrome, and in case no. 6 rhabdomyosarcoma.

Treatment prior to referral included attempts to establish the diagnosis by biopsy, tracheostomy for airway management (in one case) or attempts at removal subsequently presenting with recurrent disease. These cases highlight that incomplete removal is likely to present with recurrence later.

Treatment in these cases was required both to surgically remove the tumour in the short term, and also to treat the consequences of the mass effects of the tumour in the long term (which therefore will depend on the particular site affected).

The mass effect of the tumour affected both the underlying local skeleton (Fig. 18) and the adjacent soft tissues and may subsequently distort local growth. Structures in this series which were particularly vulnerable included the mandible (case nos. 2, 4, 5, 6, and 7), and the soft tissues in the region of the soft palate and oropharynx (case nos. 1, 2, 3, 4, 5, and 6). The consequences of the mass effect of the tumour may therefore require long-term multidisciplinary management and these cases highlight that the management following teratoma removal is dependent primarily on the site and extent of the tumour.

Tumours requiring the greatest number of further operative interventions were those tumours in sites adjacent to the soft palate and the Eustachian tube. The parapharyngeal tumour (case 1) required the most additional procedures. This was partly due to its extension into the middle ear and its effect on Eustachian tube function and partly to its effect on the soft palate, and hence speech. This in turn resulted in prolonged speech therapy after the second excision at the age of 4 years.

The tumours in the infratemporal fossa (case nos. 2 and 6) have also required additional surgical procedures following excision. These related to the palate, which has required initial reconstruction and later on repair and revision with pharyngoplasty, as growth has proceeded. During adolescence it was noted in both these cases that there was facial asymmetry and mal-occlusion. The latter was treated by orthodontic treatment alone in case 2, while case 6 is planned to have bi-maxillary surgery. In these two cases there also remained visible deformities due to the temporal hollowing.

The occurrence of intracerebral and extracranial teratomas in case no. 5 is exceptional but has already been reported (Montoya et al., 1980). Curiously, both careful intraoperative exploration and pathological as well as radiological review failed to confirm (or deny), whether it was a single or multiple lesions. Multiple teratomas in the head and neck region have also been previously reported (Dudgeon et al., 1974). However, histological examination of the tumour removed at the first operation was thought to be complete. The development of a temporary III nerve palsy is not surprising as a craniotomy was necessary to remove the tumour.

CONCLUSION

These tumours can be treated effectively and enable the child to develop into a successful adult. However, a number of different specialties are required to provide treatment including surgical interventions.

This highlights that head and neck teratomas are ideally treated in specialist centres which not only can successfully excise the tumour but can provide long-term multidisciplinary management. We would also agree with previous recommendations that those affecting the cranial base are best managed by combined Craniofacial and Neurosurgical intervention.

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Case report

The use of sentinel node biopsy in the management of epitheloid haemangioendothelioma of the lip

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Sentinel node biopsy;
Haemangioendothelioma

Summary This report describes a rare tumour, an epitheloid haemangioendothelioma affecting the lower lip. This tumour has a predilection for the head and neck region in young adults. Its potential to metastasise is well recognised, but the likelihood of this is currently uncertain. Current management is usually to locally excise the tumour and follow up, although there is a recognised risk that subsequent presentation with metastatic nodal disease can occur. We present a case occurring in the lip of an 18-year-old girl who had a sentinel node biopsy performed as a staging tool in conjunction with excision of a local recurrence. Although clinical examination and CT imaging of the head and neck found no evidence of metastatic disease, the sentinel node was found to contain metastatic tumour. The result of this unexpected finding was that she was investigated further with additional CT scanning of her chest and abdomen. Subsequently, a therapeutic modified radical neck dissection preserving the accessory cranial nerve was undertaken. After 3 years she remains well with no evidence of recurrent tumour. We believe that the consequence of undergoing sentinel node biopsy, which detected early metastatic tumour and her subsequent treatment, suggests a role for sentinel node biopsy in the management of epitheloid haemangioendothelioma.

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1. Introduction

Epitheloid haemangioendothelioma is a rare vascular tumour. It has been reported to occur in the head and neck region but particularly in sites

adjacent to the oral cavity.¹ These tumours are unusual in that they commonly occur in children and teenagers.²⁻⁵ The malignant potential of these lesions is unclear as a number of reports present different findings.²⁻⁵ Currently these tumours are regarded as malignant in view of their high morbidity and mortality, and the possibility of presentation with metastatic disease after the primary tumour has been excised is well recognised.⁵

Current treatment commonly consists of excision of the primary tumour and if there is no clinically

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detectable metastatic tumour, regular follow up in view of the recognised risk of subsequent presentation with recurrent tumour. Since many of those with this tumour are teenagers, this management protocol with its uncertainty is unsatisfactory both to patient and clinician.

We report a case of a teenager who presented with locally recurrent disease who was managed using sentinel node biopsy to stage her disease. The unexpected finding of occult metastatic disease in the regional lymph node upstaged her tumour and resulted in the alteration of her subsequent clinical management.

2. Case report

An 18-year-old woman presented to the Oral Medicine clinic with a 6 month history of a slowly growing painless swelling in her lower lip (Fig. 1). The swelling was excised and sent for histological evaluation. Histological examination identified the lesion as an epithelioid haemangioendothelioma, and the specimen margins were reported to be tumour free.

Four months later it was noted that the swelling in the lip had recurred. On examination a thickened scar in the lip mucosa could be seen, but no obviously palpable recurrent tumour. There remained uncertainty as to whether this represented recurrent tumour, so she was referred for plastic surgical assessment.

At this time the clinical appearance was of recurrent tumour so further local excision was considered. Because this swelling was thought to be recurrent tumour, the possibility of occult metastasis was also considered given the behaviour of this tumour. Sentinel node biopsy was therefore arranged at the same time as the local excision to assess the regional lymph in the neck.

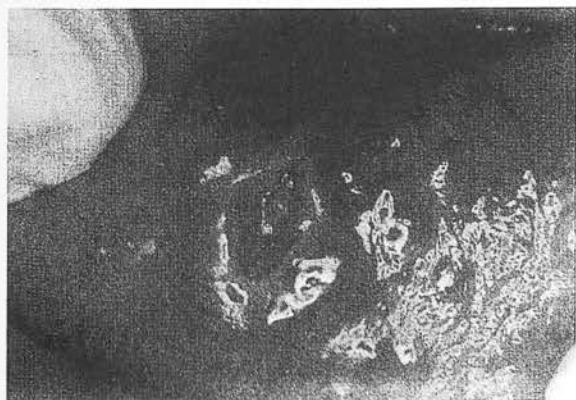


Figure 1 Photograph of the lesion at the time of initial presentation.

The day before surgery the patient attended the Nuclear Medicine Department, where up to 40MBq of Tc-99m labelled colloidal human serum albumin, Nanocoll, with a mean particle size of 80 nm was injected around the scar. Static lymphoscintigraphy was performed at 15, 30 and 60 min following injection, or until the first appearance of sentinel nodes within the neck. The position of the hot nodes was marked on the skin.

The next day during surgery, approximately $\frac{1}{2}$ –2 ml of Patent Blue V dye (Laboratoire Guerbet, Aulnay-Sous-Bois, France) was injected into the same site as radiocolloid, and skin flaps suitable for a sentinel node dissection were raised. Blue stained lymphatics were followed to blue lymph nodes (Fig. 2), and radioactivity confirmed with a Neoprobe 1500 hand held gamma probe (Neoprobe Corp, OH, USA). Radioactivity was confirmed within the sentinel node *ex-vivo*. This node was sent for routine histology where it was found to contain metastatic tumour.

Full body C.T. scanning was subsequently undertaken to try to identify further metastatic tumour, particularly in the lungs and liver, but none was detected. A modified radical neck dissection preserving the accessory nerve, the internal jugular vein and the sternomastoid muscle, on the affected side was undertaken but no further tumour was identified in any lymph nodes.

Three years later the patient is alive and well and has no evidence of recurrent tumour. The scars on the lip and the neck are maturing in a cosmetically satisfactory manner.

3. Discussion

Epithelioid haemangioendothelioma is a rare tumour but has previously been reported to occur in the lips. Earlier reports described the tumour to

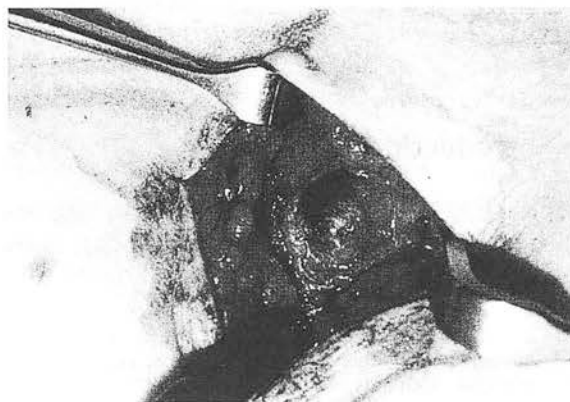


Figure 2 Close up view demonstrating the dye within the enlarged node.

be a low grade malignant vascular neoplasm.⁴ However, it is now regarded as fully malignant in view of its high morbidity and mortality.⁵ Up to 30% of cases are associated with metastasis, and the mortality rates have been reported to be 17%,⁵ but this increased to 65% if the lungs were affected.⁶ This information about the condition led to a dilemma in the management of a teenager with locally recurrent disease, where there was judged to be a risk of occult metastasis but the wish to avoid unnecessary scarring to the neck skin was also obvious.

To overcome the dilemma sentinel node biopsy was undertaken at the same time as a wider excision of locally recurrent disease. The use of sentinel node biopsy in the management of squamous carcinoma in the head and neck is undergoing evaluation.⁷ The sentinel node concept states that a tumour spreads via lymphatics to the first echelon lymph node encountered in the lymph node basin and this spread is embolic in nature. If the sentinel, or first echelon, node can be identified and examined for the presence of tumour metastases, the need to perform an elective staging lymph node dissection is more clearly defined. The concept has been mainly applied to breast cancer⁹ and malignant melanoma.⁸ In these cancers sentinel nodes free from tumour imply a regional lymph node basin free from tumour with a high degree of accuracy. Sentinel node biopsy has also been used in the head and neck region in the management of merkel cell carcinoma¹⁰ and thyroid malignancies.¹¹

The finding of metastasis in the sentinel node in this case has led to a change in the clinical management. The upstaging of the disease triggered an oncology referral and a total body CT scan. This showed no further disease anywhere but having upstaged the neck we have proceeded to perform a modified neck dissection on the affected side.

We believe that this is the first report of sentinel node biopsy in the management of epithelioid

haemangioendothelioma. It is noteworthy since the finding of occult metastatic tumour on sentinel node biopsy in this case has altered the clinical management of this case, with the consequence that to date there is no evidence of recurrent tumour. This leads us to propose that it may play a role in selected cases in the management of this tumour.

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Modified transoral approach for resection of skull base chordomas in children

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Abstract

Purpose Chordomas are rare slow growing, locally destructive tumours originating from remnants of the primitive notocord and are found most commonly in the clivus and sacrococcygeal region. These tumours usually present in early adult life but on occasion can present in childhood. The combination of the skull base location and paediatric patient size makes access to these tumours particularly challenging.

Methods and results We report a multidisciplinary technique used in two cases in children where a modified extended palatal split was undertaken to allow greater access for tumour excision.

Conclusion This approach allows for good access to the skull base region to allow for maximal tumour resection. This technique also appears to have minimal impact on palatal function and no adverse effects on the upper airway management.

Keywords Chordoma · Paediatric · Skull base · Transoral · Palatal split

Introduction

Chordomas represent 0.1–0.2% of all primary intracranial neoplasms [1, 2, 4]. They are slow-growing tumours arising from the remnants of the notochord and are found in close association with the axial skeleton. Common sites include the clivus, cervical and sacrococcygeal region. Despite their rate of growth, the extremely high local recurrence rate and their intimate relation to critical structures have often resulted in high mortality rates. These tumours usually present in early adult life but on occasion can present in childhood.

Paediatric skull base tumours are particularly challenging to access to allow for maximal surgical resection (Fig. 1). As an added challenge, chordomas are particularly difficult to treat due to their local infiltrative nature and deep location. The extent of resection has thus far been the most significant determining factor in rate of recurrence and mortality [2, 3, 6].

We report a multidisciplinary technique used in two cases in children, where an extended palatal split was undertaken to allow neurosurgical access for excision to be attempted.

Method

We reviewed the case notes of patients with chordoma to confirm that the inclusion criteria were met and to assess the postoperative progress of palatal function. Additionally, we obtained intraoperative photographs, which carefully documented the modified surgical approach using an extended palatal split for access.

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Fig. 1 Case 2—age 7 years. Pre-operative MRI

Operative technique

The submucosal layer of the hard and soft palate is infiltrated with local anaesthetic 2% lignocaine and 1:80,000 adrenaline for haemostasis. The soft palate is split midline and extended to include the anterior hard palate medial to the alveolar ridge. Mucoperiosteal flaps are then raised via blunt dissection based on the greater palatine artery to expose the hard palate (Fig. 2). Stay sutures are placed to reflect the flaps for maximal exposure. Hereafter, bone from the posterior part of the hard palate can be

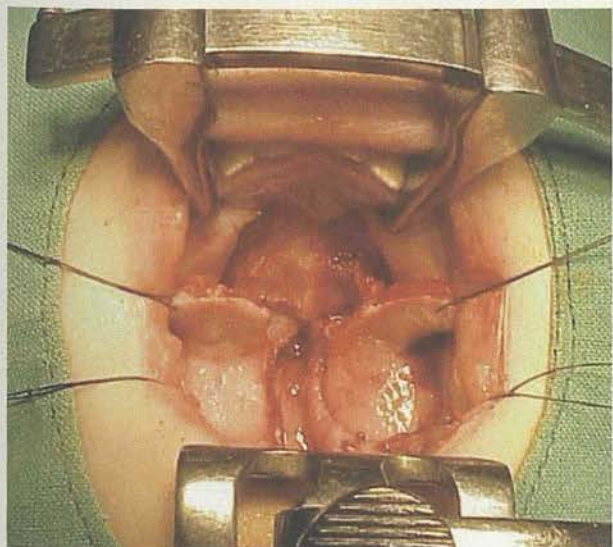


Fig. 2 Case 1—age 11 months. Palate split midline and flaps raised based on the greater palatine artery

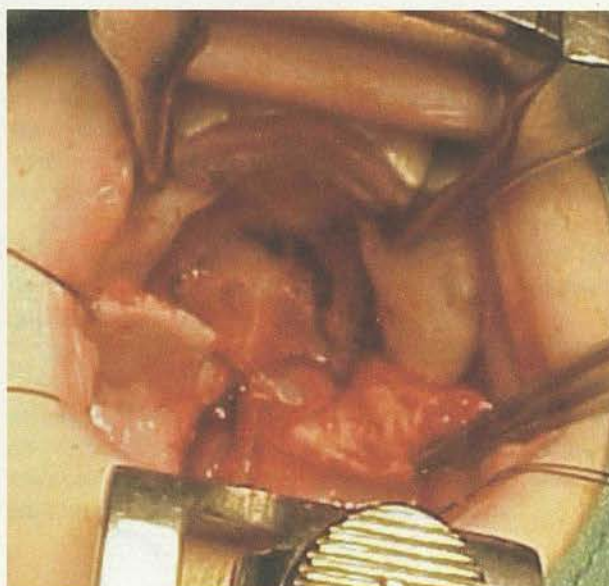


Fig. 3 Case 1—age 11 months. Bone from the posterior part of the hard palate excised to allow improved access to the tumour

excised to allow for improved access to the tumour (Fig. 3). With this level of exposure, the neurosurgeon is afforded greater access to the skull base tumour to perform the resection (Fig. 4).

Closure is performed using the Veau–Wardill–Kilner technique for cleft palate repair as described by David et al. [7]. The stay sutures are released and the repair performed in layers. Nasal mucosa is closed with 4-0 Vicryl Rapide,



Fig. 4 Case 1—age 11 months. Maximal exposure to the skull base region



Fig. 5 Case 1—age 11 months. Healed palate at 4 months post-operation

muscular layer with 3-0 Vicryl Rapide and finally the oral mucosa with 4-0 Vicryl Rapide.

Results

Two patients (ages 11 months and 7 years) underwent surgical resection using this modified approach. Palatal function was restored in both cases with excellent wound healing (Fig. 5). The older child required speech therapy in the first year with good results, and we are continuing to follow his progress. The younger child did not require speech therapy but died at the age of 5 years from complications secondary to adjuvant therapy.

Discussion

Traditional approaches vary from cranio-orbitozygomatic, maxillotomy and transthemoidal through to transcondylar

and transoral routes [4]. Samii et al. [5] published a retrospective study of 49 patients who had undergone resection of skull base chordomas at a single institution and reported that their three most frequently used approaches were transthemoidal (36.3%) followed by pterional (23.4%) and retrosigmoid (23.4%).

Colli and Al-Mefty [6] reviewed 53 patients with chordomas of the craniocervical junction and reported that the cranio-orbitozygomatic approach was most frequently utilised at their institution.

Our approach provides excellent exposure of the skull base region. This maximises the potential towards total resection, which is critical in the long-term prognosis of chordomas. In addition, this approach also appears to have minimal long-term effects on palatal function and no adverse effects on the upper airway management.

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SHORT REPORTS AND CORRESPONDENCE

Differential diagnosis of a soft tissue mass following long term parenteral iron injection

Prolonged use of soft tissue injections of iron-carbohydrate complexes for the treatment of iron deficiency anaemia are known to induce calcification with prolonged use, particularly when a single injection site is used.¹

A 66-year-old female had been treated by injection of iron polymaltose into the soft tissues of her right buttock monthly, for a period of 8 months, for the treatment of iron deficiency anaemia. A hard lump soon developed in the injection site and over the next few years eroded through the skin. Imaging demonstrated that the mass was limited to the subcutaneous tissues with no intramuscular extension (Fig. 1). The area was excised and a vacuum-assisted closure (VAC) dressing applied. The defect was subsequently closed with a split-thickness skin graft. Subsequent histology revealed siderosis and inflammatory change.

Soft tissue injection of iron-carbohydrate compounds has been used for the treatment of iron-deficiency anaemia since 1955.² Modern drug therapies have now reduced the requirement for intramuscular iron administration. Soft tissue iron-carbohydrate injections are known to cause calcification of the soft tissues at the injection site. In addition, there is some evidence that long-term therapy can induce soft tissue sarcoma formation. The data available are not yet strong enough to show a causal relationship in man.^{2,3} However, we suggest that malignancy should be excluded in a soft tissue mass following parenteral iron treatment, using imaging and soft tissue biopsy.

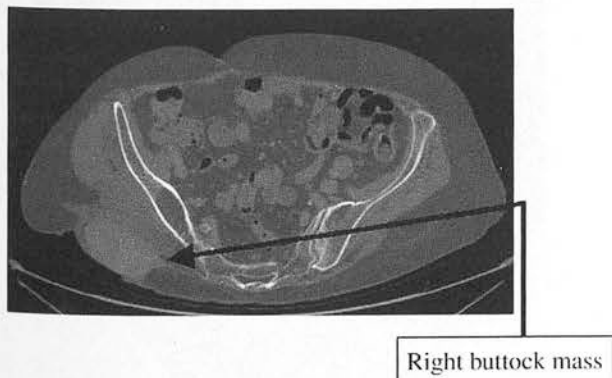


Figure 1 Axial CT slice at mid-sacral level demonstrating a soft tissue mass.

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Rhabdomyosarcoma of the mandible – long term management from childhood to adulthood*

Tumours of the head and neck in infancy are rare but rhabdomyosarcoma is one of the most common soft tissue sarcomas in infants and children. It has a predilection for the head and neck region.¹ The current treatment of a combination of ablative surgery, chemotherapy and radiotherapy has dramatically improved the long term survival rates over the last 20 years.² Approximately 65% of children diagnosed with rhabdomyosarcoma will survive after current multimodality treatment.³ The 5 year overall survival is reported to be greater than 80% in tumours that are either completely resected or grossly resected with only microscopic residual disease.⁴

This combination treatment regimen does, however, have significant implications for long term reconstruction and rehabilitation as the patient progresses from childhood through to adulthood. The reconstructive process begins with the initial surgical defect but, as the child grows, the effects of the high dose radiotherapy on the immature facial skeleton begin to manifest themselves and continue

* Presented at the Australia and New Zealand Head and Neck Conference, Wellington, New Zealand. October 2006.

to do so, both locally and systemically, throughout the developing years up to skeletal maturity and adulthood.

A multidisciplinary approach is therefore needed to treat and care for these patients from childhood to adulthood.

Case report

This is illustrated in the case of a 4-year-old boy who presented to his general practitioner with a swelling on the right side of his mandible. A biopsy of the lesion was reported as rhabdomyosarcoma arising within bone. Preliminary treatment involved intra-arterial chemotherapy using adriamycin, cyclophosphamide, vincristine and actinomycin D for 7 days. This was followed by chemotherapy for 2 years. There was a good response to the initial chemotherapy and he immediately progressed to having external beam radiotherapy to the right side of the face. He then underwent a right hemi-mandibulectomy as part of his oncological treatment programme. He has remained disease free ever since, with no recurrence of the original tumour.

The patient first attended the Australian Craniofacial Unit at the age of 11 years, some 7 years after the completion of his combination tumour treatment. At presentation there was gross hypoplasia of the soft tissues of the right cheek and right maxilla and zygoma. As a result of the hemi-mandibulectomy there was loss of mandibular contour and the chin point was deviated to the right (Figs. 1 and 2).

At 13 years of age he underwent a Le Fort 1 maxillary advancement osteotomy to correct the midface hypoplasia and a left subsigmoid mandibular osteotomy to centralise the mandible. A free DCIA (Deep Circumflex Iliac Artery) osseocutaneous flap was used to reconstruct the mandible

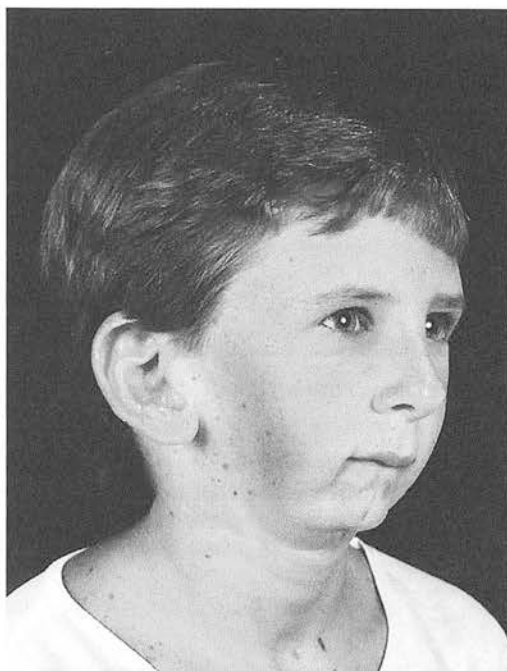


Figure 2 Age 11 years—7 years post chemotherapy, radiotherapy and surgery.

and augment the soft tissues of the cheek. The skin paddle was de-epithelialised and trimmed as necessary to contour the cheek. The block of iliac crest bone was used to reconstruct the mandible and was approximately 6×2 cm. The vessels were anastomosed end-to-end to the ipsilateral facial artery and vein. The temporomandibular joint was reconstructed with a costochondral rib graft. The senior author (DJD) has extensive experience with the use of costochondral rib grafts in the reconstruction of temporomandibular joints in patients with hemifacial microsomia, for example, and they have proved to be very reliable in terms of their function. Onlay bone grafts from the iliac crests were used to augment the right zygoma and chin (Figs. 3 and 4). Although this gave him a good initial cosmetic result, the onlay bone grafts have a very limited growth potential and there is a degree of resorption with time. Therefore, over the years as he grew into adolescence, he required further procedures to augment the right side of the face. These took the form of further onlay bone grafts, dermofat grafts and, on one occasion, tissue expansion was used to address some soft tissue puckering in the lower right lip/cheek region. The insertion of the tissue expander was technically challenging due to the abundant fibrotic scarring and tethering of the tissues within the cheek. The expander was placed superficial to the de-epithelialised DCIA flap. Tissue expansion was carried out over a relatively short period of time, 4 weeks, in an attempt to minimise any detrimental effects to the underlying onlay bone grafts. Nevertheless, adequate tissue expansion was achieved resulting in a satisfactory cosmetic outcome. Although the remaining teeth appeared sound on intra-oral examination, a radiograph demonstrated severe underdevelopment of the teeth roots as a result of the radiotherapy (Fig. 5). He gradually lost

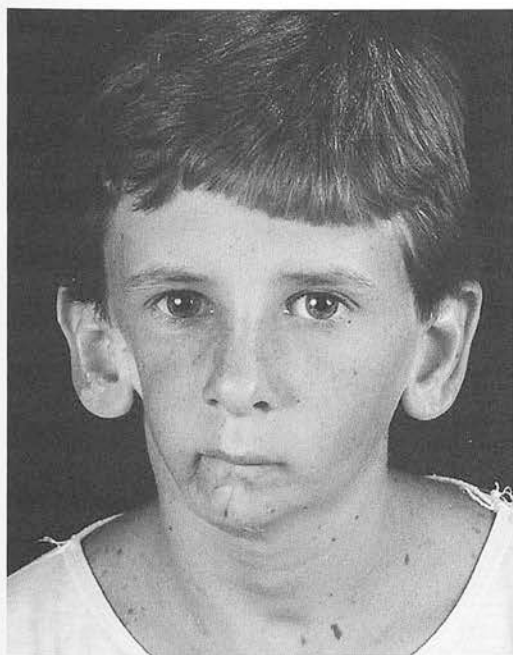


Figure 1 Age 11 years—7 years post chemotherapy, radiotherapy and surgery.

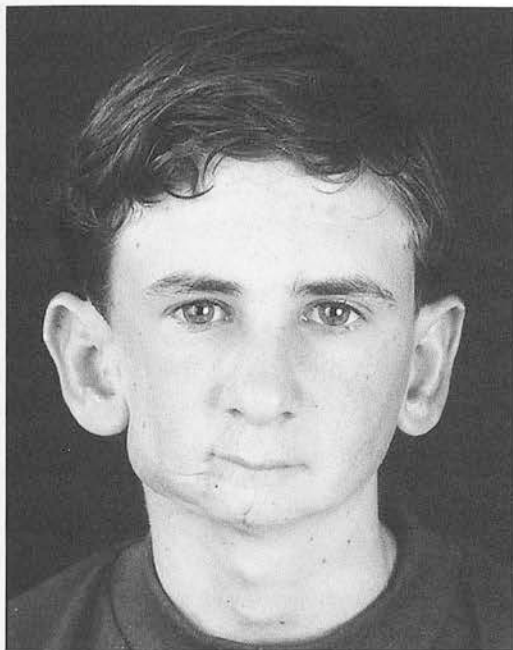


Figure 3 Age 13 years — after reconstruction.

all of his teeth due to premature exfoliation and by his 20's he was edentulous and required full oral rehabilitation in the form of osseointegrated retained dental implants. Similarly, placement of the implants was difficult due to restricted access as a result of the scarring of the perioral tissues. Fortunately, wound healing and osseointegration of the titanium implants was not a problem and full upper and lower implant retained teeth were fitted. Figures 6 and 7 show him at 28 years of age (present day).

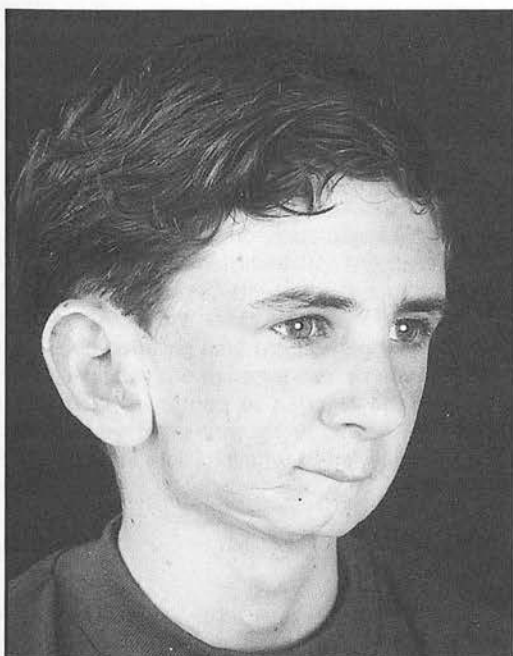


Figure 4 Age 13 years — after reconstruction.

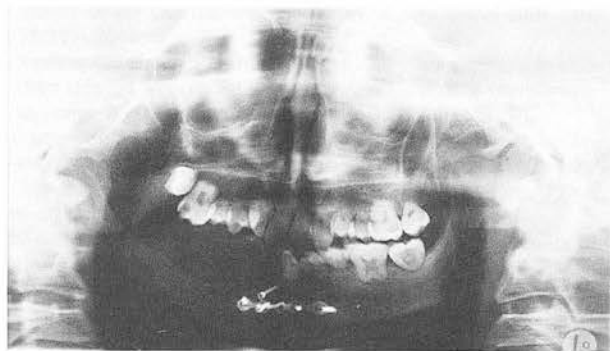


Figure 5 Poorly formed dental roots.

At 28 years of age he underwent fine needle aspiration of a nodule in the right lobe of the thyroid. This was reported as 'not significant' but he remains under surveillance for thyroid malignancy, again, as a result of the radiotherapy.

Discussion

The use of multidrug chemotherapy and external beam radiation followed by surgery has resulted in a dramatic increase in survival for those children with rhabdomyosarcoma.² Over the last 25 years, when this patient first presented, the role of surgery in the management protocol of children with head and neck rhabdomyosarcoma has undergone a radical transformation. The advancement in craniofacial reconstruction techniques and free tissue transfer have allowed for immediate reconstruction of large resection defects with acceptable functional and cosmetic results, even in irradiated areas. However, this multimodality treatment regimen does result in serious sequelae both local to the tumour site and systemically. The

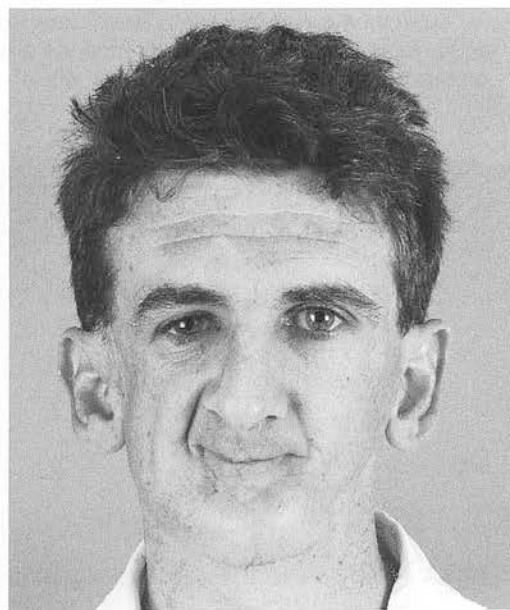


Figure 6 Age 28 years — 'final' result.

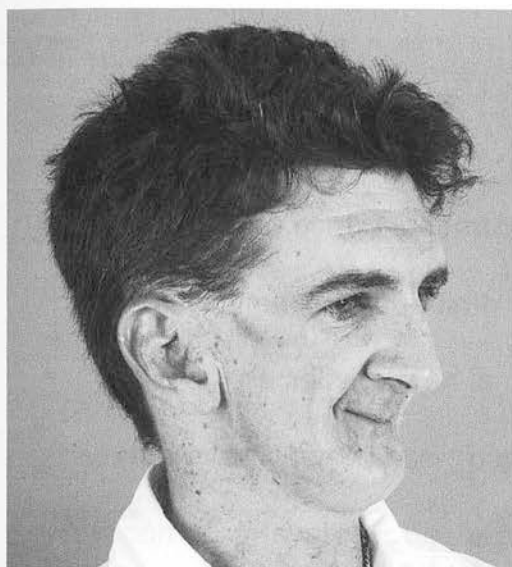


Figure 7 Age 28 years — 'final' result.

excellent survival rate with multimodality treatment has allowed the follow up and observation of these patients for many years and has given clinicians the opportunity to address the effects of treatment.² One of the most important concerns in paediatric radiation oncology is optimising quality of life while attempting to cure a life-threatening malignancy. The use of external beam radiotherapy in the head and neck region in children has many possible long term effects. These include arrest of growth of the immature craniofacial skeleton, damage to normal tissues and organs, systemic neuroendocrine dysfunction, cognitive defects and dental developmental abnormalities.⁵

Recently, radiation techniques have been modified, such as improved beam shaping, hyperfractionated doses and reduced doses to surrounding healthy tissues.⁵ In selected cases brachytherapy can be used for localised radiotherapy control instead of external beam radiation. The brachytherapy is in the form of iridium-192 wires embedded in rubber (gutta percha) which is then placed in the wound bed thus avoiding radiation exposure to more distant sites.⁶

In conclusion, over recent years significant advances have been made in the multimodality treatment of rhabdomyosarcoma in children. However, the challenge is to maintain excellent cure rates whilst at the same time reducing the devastating long term sequelae of the treatment — in particular the external beam radiotherapy. We recommend that this group of patients are managed by a multidisciplinary team throughout life so that the long term effects of their multimodality treatment as a child can be monitored.

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Elastofibroma dorsi: a rare diagnosis in chest wall tumours

A 63-year-old financial director presented with a right-sided lump around his scapula that had caused discomfort for 5 months. He also noted a smaller one on the left in an identical site. These swellings became noticeable when he abducted his arms (Fig. 1). He was a serious archer and golfer. In the past he had had a pectus procedure. The

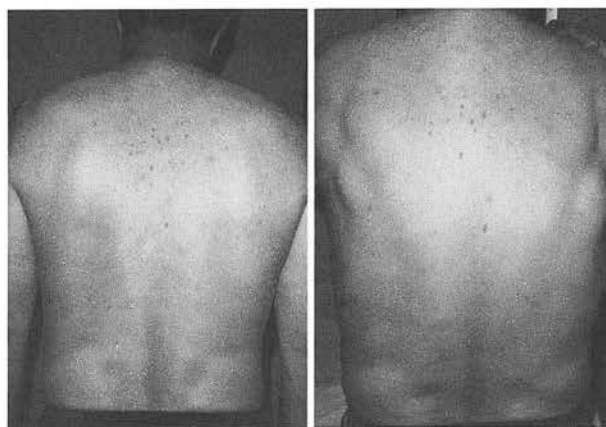


Figure 1 The patient's back with arms at rest and then abducted, exposing the bilateral subscapular tumours.

CHAPTER FIVE

INTRODUCTION

Pathological processes which affect the skeleton and striated musculature of the locomotor system are particularly important in relation to their impact on the limb structure and function, while their impact on the craniofacial skeleton is often less of a clinical priority.

However, as these papers demonstrate diseases affecting the locomotor system do affect the growth and development of the craniofacial region in children, leading to morphological changes. These and can be difficult to manage in their own right, and as well as managing them as part of co-ordinated care with rheumatologists and orthopaedic surgeons, who are themselves managing systemic or local complications of the conditions elsewhere.

CHAPTER FIVE - PAPERS

The first paper is case report of cousins with Parry-Romberg disease. This degenerative condition of starts in childhood and the aetiology is unknown with cases thought to be sporadic. This case report is the first reported familial example of this condition. This family has a complex genetic relationship, the fathers are twins and the mothers are sisters, so they share many common genes. This example could be a chance finding but raises the possibility that the affected individuals may have familial predisposition to this curious condition.

The second paper is collaborative study with Plastic surgeons in Indonesia to review children with pterygium syndrome who either had localised manifestations or associated craniofacial anomalies occurring in association with pterygium elsewhere in the skeleton. This paper demonstrates a wide range of anomalies and demonstrates the role of reconstructive surgery to improve function and activities of daily living.

The third paper is a case report describing the clinical features of a child with nemaline myopathy. This is a rare condition but the abnormally weak facial musculature results in characteristic dysmorphic changes in the facial skeleton. The management with bimaxillary orthognathic surgery to improve the facial appearance is described along with the specific precautions of safely administering an anaesthetic to an affected individual which are also reviewed.

The fourth paper is a case report highlighting a late complication of mandibular distraction in the immature facial skeleton in a child with pterygium syndrome, the aim of surgical treatment to remove his tracheostomy. This case demonstrated that the immature permanent tooth roots were simultaneously distracted along with the mandible and ultimately were almost double their normal length. This necessitated surgical removal. This case highlights a potential complication of distraction osteogenesis of the mandible in children, when osteotomies are undertaken close to developing tooth germs.

The fifth and final case is a collaborative study with the Ophthalmology department investigating the long term visual outcomes in patients with fibrous dysplasia. This condition often begins and is diagnosed in childhood although surgical intervention may not be required until after skeletal maturity has been reached. The management of the disease around the optic canal is a controversial topic with some clinicians advocating surgical decompression while other clinicians recommend a strict conservative management course. This study reviewed a cohort of patients many of whom were first diagnosed in childhood, and the findings suggested that optic canal decompression can help preserve vision in severely affected individuals.

In summary this collection of papers describes some previously unreported clinical features, highlights management challenges and in the last paper reviews the long term outcome of an important clinical dilemma for craniofacial surgeons.



CASE REPORT

Familial Parry–Romberg disease

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KEYWORDS

Parry–Romberg
disease;
Face;
Hemifacial atrophy

Summary Parry–Romberg disease (or hemifacial atrophy) is a rare condition affecting the face. It commences in childhood but its aetiology remains unknown, and is sporadic. Two cases are presented who were biological first cousins. We believe that this is the first recorded example of this condition occurring in family members.
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1. Introduction

Parry–Romberg disease (or hemifacial atrophy) is a curious clinical condition with its peak incidence during childhood and teens. It commonly affects the fronto-parietal area and may extend inferiorly to affect the facial skeleton and skin on the affected side of the face. The localised atrophy produces facial asymmetry and the characteristic “coup de sabre” deformity of the forehead. It was originally described by Parry in 1825 [1] and later by Romberg in 1846 [2], the names being combined later.

The aetiology remains uncertain and treatment is aimed at reconstruction once the condition has stabilised or “burnt out”. To date all reported cases have been sporadic, however the following case reports are of two cases in who were family members.

2. Case reports

2.1. Case 1

A 9-year old boy who had previously been fit and well developed a pale lesion on his left cheek. He gradually developed associated muscle weakness of the adjacent left lip associated with anaesthesia of his top lip. There was no lymphadenopathy. Three years later he developed a similar patch on his right cheek. Excision was undertaken and histological examination was unremarkable apart from a perivascular lymphocytic infiltration. No organisms were seen and tuberculosis and leprosy were excluded (Fig. 1).

Subsequent review by dermatology at age 13 years diagnosed the left cheek lesion to be morphea, while lesion right cheek was diagnosed as pityriasis alba. This right lesion resolved with topical medication. Review 1 year later found that he had progression of the atrophy affecting his left cheek leading to revision of his diagnosis to Romberg disease. He was then referred for craniofacial assessment.

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Fig. 1 Case 1 age 16 years demonstrating facial asymmetry.

On examination he was noted to have soft tissue wasting with disturbance of his dentition producing an upward occlusal cant but both the fifth and seventh cranial nerve function was normal. He was noted to have recurring migraneous headaches,

which have now reduced in frequency. Haematological examination revealed an eosinophilia along with a low C4 count, however his serology was negative for antinuclear factor (ANF) (Fig. 2).

He declined soft tissue reconstruction at that time but remains under regular review although it appears the condition has now "burnt out".

2.2. Case 2

A 14-year old girl who was the cousin of case 1 and who had previously been fit and well, was noted to develop a depression on her right forehead which extended into her neck. Over the following 2 years the right side of the face failed to develop as much as her (unaffected) left side and her face became increasingly asymmetrical. At the same time she

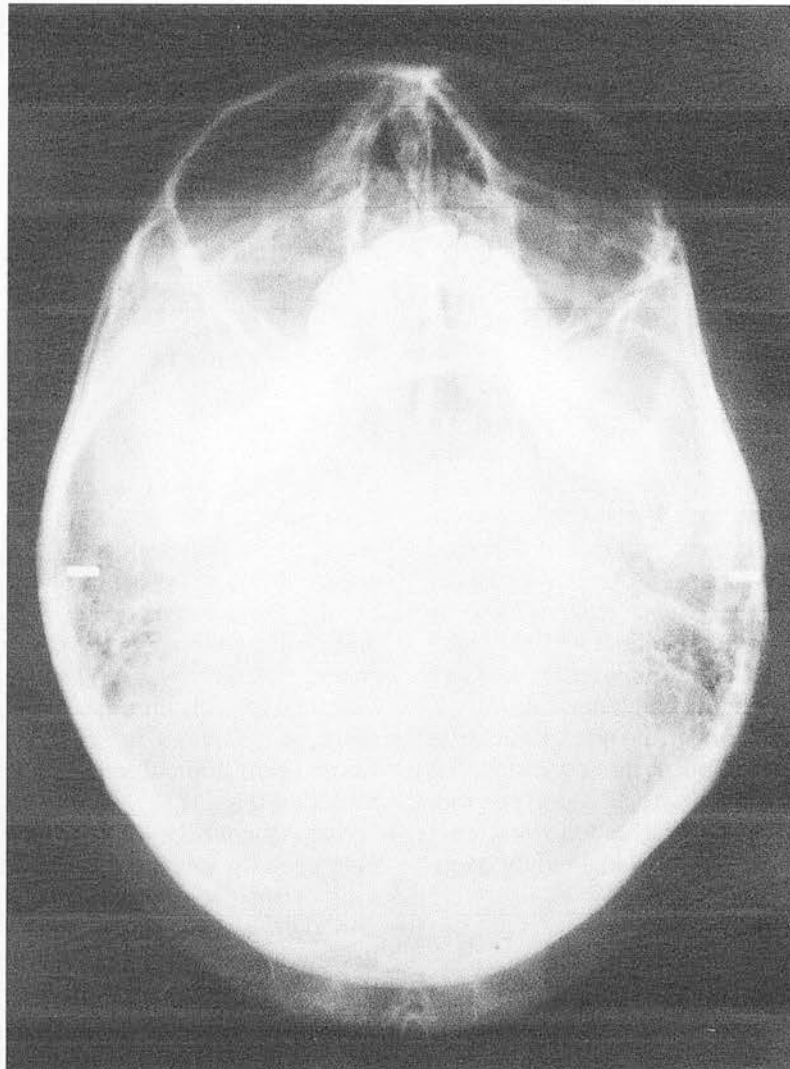


Fig. 2 Posterior-anterior facial view of case 1 demonstrating the asymmetry of the mid-face skeleton and the occlusal cant on the affected side.



Fig. 3 Case 2 age 16 years demonstrating facial asymmetry.

had an increasing number of migraneous headaches. She was referred for craniofacial opinion (Fig. 3).

On examination she was noted to have a “coup de sabre” deformity with thinning of the skin and fat of the right cheek. The right temple hair was also thin. The underlying facial skeleton was also affected with right sided hypoplasia of the maxilla and mandible producing a malocclusion with an occlusal cant (Fig. 4).

The haematological investigation revealed her to have an eosinophilia but her serology was positive for ANF. Currently, she is considered to still have active disease but once this is stable will be offered reconstruction with bi-maxillary orthognathic surgery and soft tissue reconstruction with free tissue transfer to correct her asymmetry.

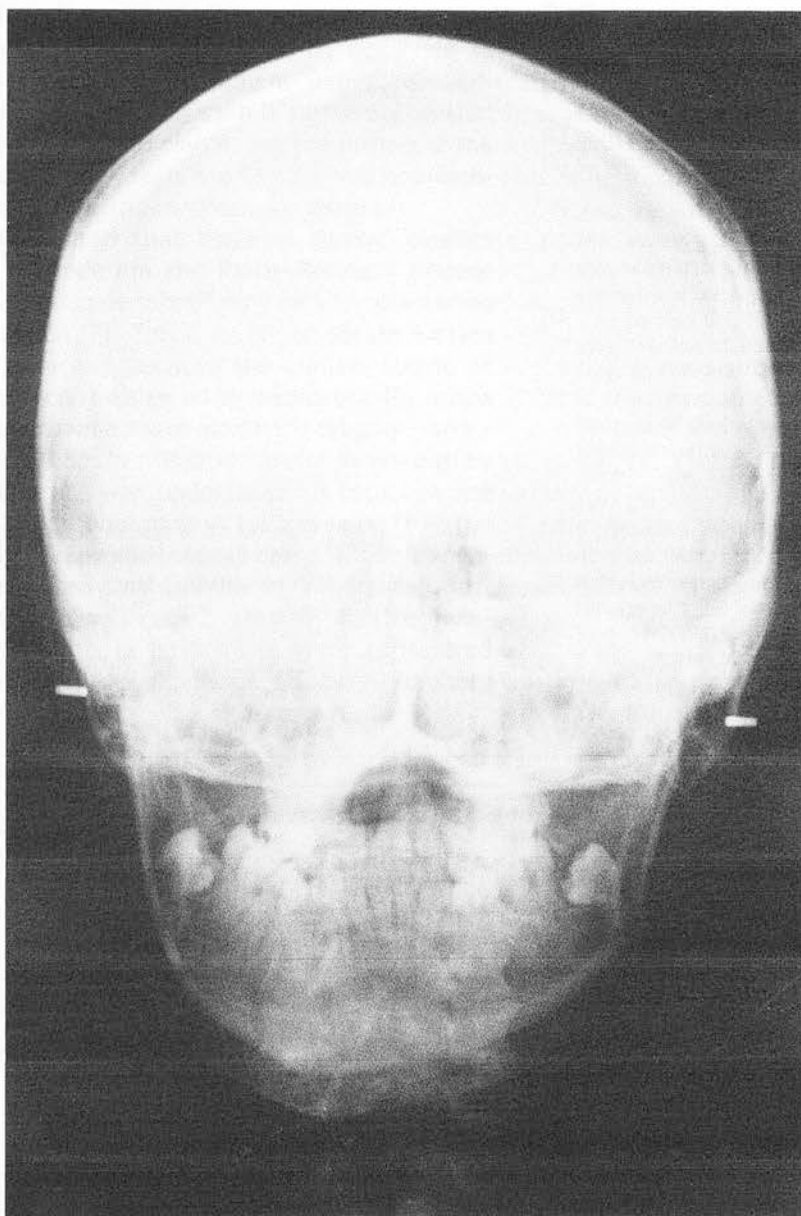


Fig. 4 Occipito mental radiograph of case 2 demonstrating the distorted bone contour in the Frontal region producing the coup de sabre deformity.

3. Discussion

Parry–Romberg disease is an acquired progressive hemifacial atrophy of the facial subcutaneous and skeleton of unknown aetiology and pathogenesis. A range of otorhinolaryngological complications affecting different structures have been described [3]. It usually presents in childhood, and the earlier the onset the greater the subsequent skeletal involvement [4]. The aetiology remains uncertain but trigeminal lymphocytic neurovasculitis has also been reported on histological assessment of tissue specimens (as occurred in case 1), suggesting a chronic cell mediated injury [5], while hyperactivity of the brain stem sympathetic centres has also been proposed as a cause [6].

The occurrence of the condition occurring in members of the same family is unprecedented. The family history in this case is significant in that not only are these two cousins but the fathers of the two affected individuals are themselves non-identical twins, while the mothers are sisters.

It is recognised that there is clinical overlap between scleroderma and Parry–Romberg disease and that a “coup de sabre” may be a manifestation of scleroderma [7]. This is an important distinction to make clinically because the clinical course of scleroderma can be altered by medication [8], while there is no known disease modifying drug for Parry–Romberg disease. In this case careful evaluation by clinical genetics was undertaken to establish the diagnosis. Additional support for this came from the haematological profiles of both cases. These demonstrated a raised eosinophilia in both cases, but differed in that only case 2 was ANF positive; however the profiles in both cases were considered consistent with the diagnosis of Parry–Romberg disease.

Reconstruction is normally reserved once the condition has stabilised and “burnt out”, and the goals are restoration of the skeleton and soft tissue contours to match the unaffected side [4,9]. In these cases, case 1 is still considering whether to undergo reconstruction with a free dermofat graft, while case 2 is awaiting stabilisation and surgery in the form of bi-maxillary orthognathic surgery and dermofat grafts is planned.

In conclusion two cases of familial Parry–Romberg disease are presented which is the first time this has been reported.

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Spectrum of Features in Pterygium Syndrome

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Pterygium syndrome is a complex and rare congenital deformity that consists of contractures involving multiple flexural surfaces and associated craniofacial anomalies. It often has associated conditions, including anomalies of the cardiovascular, respiratory, gastrointestinal and genitourinary systems. It may present in different forms, including multiple pterygium syndrome of Escobar, lethal multiple pterygium syndrome, popliteal pterygium syndrome, lethal popliteal syndrome (Bartsocas-Papas syndrome) and arthrogryposis multiplex congenita. The clinical presentation, multidisciplinary management and the long-term outcome in three patients with this condition are presented. [*Asian J Surg* 2006;29(2):104-8]

Key Words: congenital deformity, pterygium syndrome

Introduction

Pterygium syndrome has a wide spectrum of presentation. Multiple pterygium syndrome (MPS) of Escobar is an autosomal recessive malformation consisting of growth retardation, multiple pterygia involving neck, fingers, antecubital, popliteal, intercrural and craniofacial anomalies. The typical craniofacial features are downslanting palpebral fissures, epicanthal folds and small mandible. This syndrome is clearly distinguished from popliteal pterygium syndrome (PPS) that classically involves contractures of the lower extremities with associated craniofacial and genitourinary anomalies. PPS is autosomal dominant with variable expressivity and incomplete penetrance. The incidence of all forms of pterygium syndrome is uncertain and they are frequently nonspecifically termed as arthrogryposis.¹ There have been previous reports of the entity, but most are individual case reports.^{2,3} A highly variable presentation of this anomaly has been reported with a wide range of severity. A very severe form is the lethal MPS and lethal PPS with a high incidence of fetal death, and it is presumed that the pterygia may result from embryonic onset of fetal akinesia.⁴ Other theories such as abnormal collagen and aplasia of developing muscle fibres have also been pro-

posed.^{2,5} We reviewed the clinical presentation of three cases of this group of anomalies managed from early childhood, and report on the outcome of the multidisciplinary management of this condition.

Case reports

Case 1

A 3-year-old European male presented to the Australian Craniofacial Unit (ACFU) with MPS, Pierre Robin sequence and undescended testes. An antenatal ultrasonography at 20 weeks' gestation had reported multiple pterygium, cleft palate and cystic hygroma. Fetal distress led to an emergency caesarean section at full term.

The clinical features included extensive pterygia of the neck extending from the chin to the sternum with the chin tethered to the chest. The contractures involved all the joints of the upper and lower limbs; deformities included multiple syndactyly, intercrural pterygia, congenital hip dislocation, and rocker bottom feet (Figures 1 and 2). He was unable to walk, had difficulties using the wheel chair, and was unable to lie supine. The facial anomalies included micrognathia with restricted mouth opening, cleft of the soft palate, hypoplastic

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tongue, enophthalmos, bilateral epicanthal folds and anti-mongoloid slant (Figure 3). Other anomalies present included chest wall deformity with hypoplastic nipples and thoracolumbar scoliosis. Detailed radiological investigations revealed fusion of the cervical spine at the C1 and C2 level, and incomplete fusion of C3-4 vertebrae. The mandible was hypoplastic with an obtuse gonial angle and hyperplastic coronoid process extending into the temporal fossa (Figure 4).

A multidisciplinary team that included specialists in craniofacial, paediatric and orthopaedic surgery, paediatric and respiratory medicine, speech pathology and physiotherapy undertook management.

His neonatal period was complicated by the presence of airway obstruction and feeding difficulties. These were managed conservatively by the speech pathologist and respiratory physician. His ventilation was improved with the use of continuous positive airway pressure. Following this, at the age of 3 years, a review by the respiratory physician and formal polysomnogram revealed significant episodes of airway obstruction. He underwent linear and angular distraction of the mandible (Figure 5) to improve his airway, feeding, mouth opening and permitted repair of the cleft palate. Subsequently, at the age of 10 years, he underwent multistaged surgical release of the neck contracture with the use of split thickness skin graft in addition to several orthopaedic procedures for his lower limb anomalies that included congenital hip dislocation, fixed deformity of the hip, knee contracture

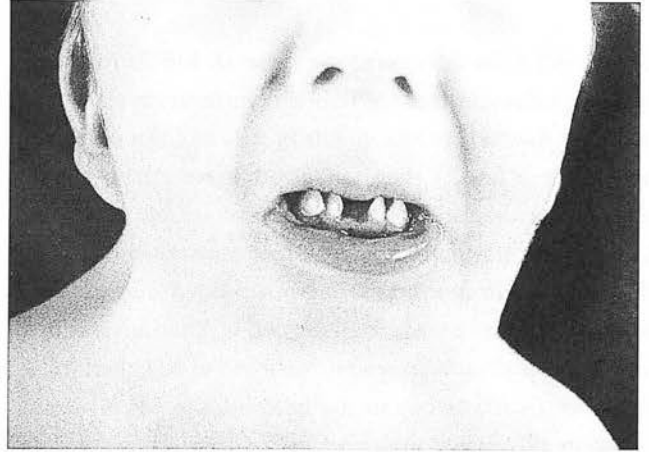


Figure 3. Case 1 at the age of 3 years showing severe neck pterygium and restricted mouth opening.

and rocker bottom feet. He is currently 12 years old, uses a wheel chair and has no trouble sleeping in the supine position (Figure 6). He will remain under multidisciplinary review until he reaches skeletal maturity.

Case 2

A European female patient was born with bilateral cleft lip and palate (CLP) with lower lip pits, popliteal contracture extending down to the ankle, preaxial polydactyly, simple syndactyly, calcaneovalgus deformity and hypoplastic labia majora. She was diagnosed with PPS. She underwent multidisciplinary assessment by a team that included specialists in craniofacial and orthopaedic surgery, orthodontics, den-

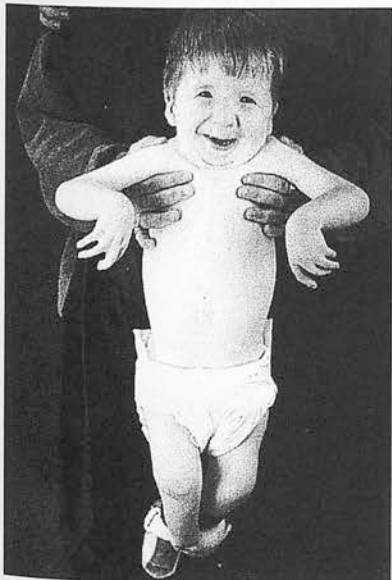


Figure 1. Case 1: 3-year old male with multiple pterygium syndrome of Escobar.

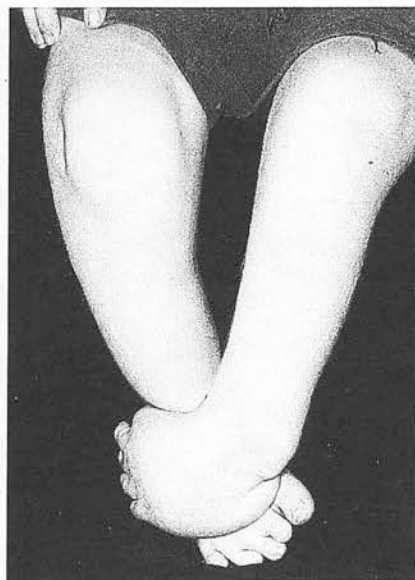


Figure 2. Case 1 at the age of 3 years showing the rocker bottom feet deformity.

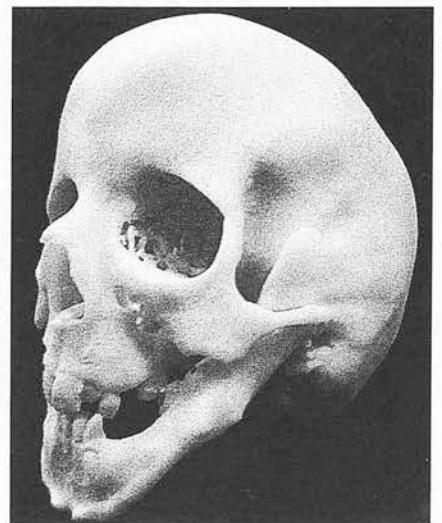


Figure 4. Nylon model of Case 1 reconstructed at the age of 3 years illustrating the mandibular deformity, particularly the hypertrophied coronoid process.

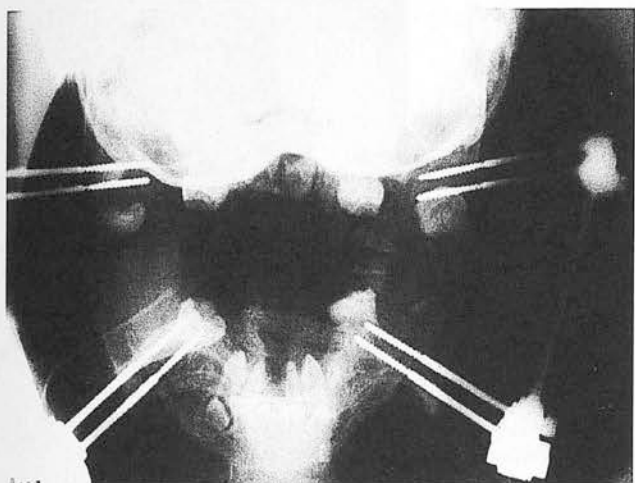


Figure 5. Plain X-ray of the mandible showing distracted mandible in Case 1 at the age of 3 years and 2 months.

tistry, speech pathology and physiotherapy. The CLP was managed according to the published cleft protocol of the ACFU.⁶ The cleft lip was repaired at 3 months and the palate at 9 months of age. A bilateral alveolar bone graft was performed at 11 years, and bimaxillary surgery and augmentation of malar bone at the age of 18 years. Final surgery included revision of the lip and augmentation rhinoplasty at the age of 20 years (Figure 7). The orthopaedic and physiotherapy team managed her knee and ankle deformities conservatively during her early childhood with splints for 2 years and achieved total correction without any residual functional deformity.



Figure 6. Case 1 at the age of 10 years showing good mandibular growth and neck contour.

Case 3

A 3-month-old Asian male presented with PPS and a range of associated anomalies. The facial anomalies included complete midline cleft of the lip and palate with nasal deformity, ectropion of the upper eyelids, frontal hirsutism and bilateral choanal atresia (Figure 8). The associated upper limb anomalies were complete syndactyly of the second and third fingers, hypoplastic thumb, agenesis of the fifth digit of the right hand, and agenesis of three radial digits of the left hand. The lower limb deformities included bilateral popliteal pterygia, bilateral talipes equinovarus, intercrural webbing and agenesis of the lateral three digits (Figure 9). In addition, he had associated genitourinary anomalies that included bifid scrotum, micropenis and testicular agenesis.

Due to the multitude of anomalies, he underwent a multidisciplinary assessment. A radiological study detailed the skeletal anomalies and genetic testing revealed chromosome 14/5 translocation (Figure 10).

Clinically, the priority was management of bilateral ectropion of the upper eyelids that was causing damage to the cornea. This was treated by release with full thickness skin grafting. A large defect in the lip and nasal structures necessitated recruitment of tissues from the cheeks and forehead using tissue expansion (Figure 11). It is planned that he will undergo further coordinated reconstructive surgery for his hand, lower limbs and genitourinary anomalies. He is presently 3 years old and will remain under review until skeletal maturity.

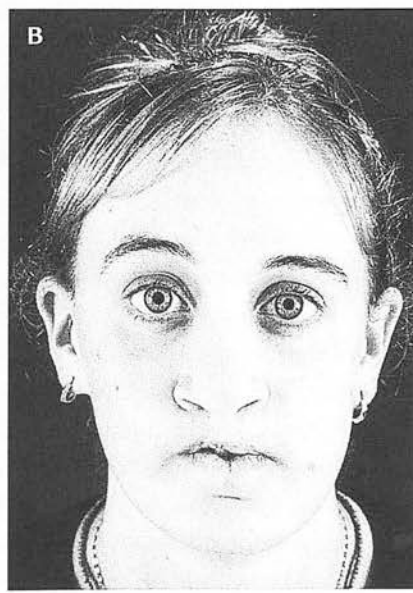


Figure 7. Case 2 with popliteal pterygium syndrome at the age of: (A) 22 years, after protocol-based management of bilateral cleft lip and palate but before orthognathic surgery, and (B) 1 year after orthognathic surgery and soft tissue refinement.



Figure 8. Case 3: 3-month-old male patient with popliteal pterygium syndrome showing multiple facial anomalies including complete midline cleft of lip and palate, nasal deformity, and ectropion of the upper eyelids.



Figure 11. Case 3 at the age of 4 months showing tissue expansion of the forehead and both cheeks for upper lip and nose reconstruction.

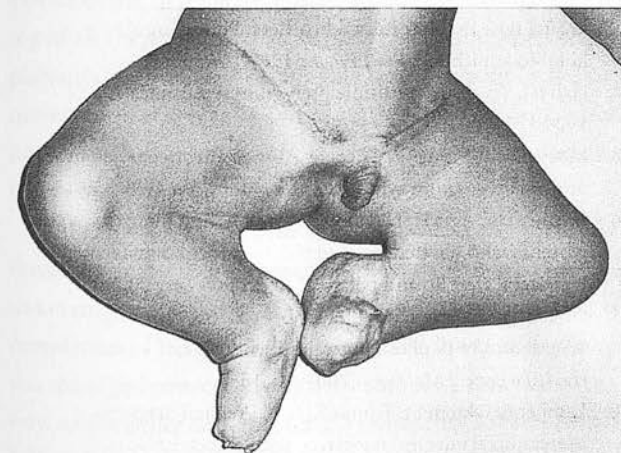


Figure 9. Case 3 at the age of 3 months showing severe pterygia extending from the groin to the heel, talipes equinovarus of both feet, agenesis of digits in both feet, and genital anomalies including absent scrotum and micropenis.

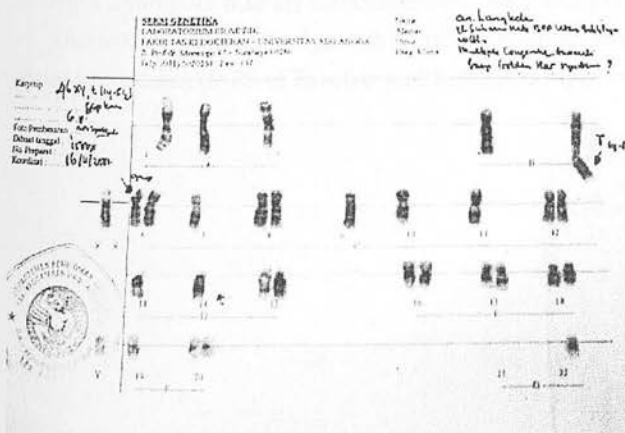


Figure 10. Chromosomal analysis for Case 3 shows: 46,XY, t(14;5)(5p) gap 6p nonspecific.

Discussion

MPS is a complex congenital deformity that consists of contractures of multiple flexural surfaces, anomalies involving the craniofacial region and extremities, and which often has associated systemic abnormalities. This condition may occur sporadically and is peculiar in that it may have autosomal dominant or recessive inheritance.

The genetic abnormality has not been detected for MPS, but a mutation in the *IRF6* gene is the possible cause of PPS.⁷ The underlying pathogenesis is not clear, but it has been proposed that it is due to decreased fetal movement.^{4,5} A prenatal diagnosis can often be established with Escobar syndrome as in Case 1 of this report, and the presence of associated cystic hygroma is one of the typical signs of MPS.^{3,8} One report mentions that the severity of the disease may be indicated by the presence of a spinal anomaly.⁹

Pterygium syndrome has a wide range of clinical presentations, and pterygia is consistently present involving several joints. PPS has characteristic involvement of the popliteal region, which may range from mild contracture as in Case 2 to extensive knee, ankle and crural pterygia as in Case 3. It is suggested that the presence of at least three of the following deformities including cleft lip and palate, popliteal pterygium, paramedian lower lip sinuses and genital anomalies are required for the diagnosis of PPS.¹⁰ Lower lip pit was seen in both our cases with PPS, and one of the reports indicates its incidence as 56% of PPS cases.¹¹ Another report suggests that PPS and van der Woude syndrome are allelic and results from mutations in the *IRF6* gene.⁷ On the other hand, MPS primarily consists of multiple contractures involving all the joints

of the limbs and the neck as well. There are significant musculoskeletal anomalies and growth retardation.^{3,9} Associated craniofacial anomalies in MPS include micrognathia, cleft palate, low set ears, antimongoloid slant, and epicanthal folds. In MPS, primary pathology may be the pterygium, and the facial and limb anomalies may be secondary to the contractures, while in PPS, all the anomalies appear to be part of the multisystem involvement. The presence of hypertrophied coronoid processes in Case 1 in this series is a consequence of the force exerted by the temporalis muscle during mouth opening.

The range of associated anomalies leads us to undertake a multidisciplinary team approach. Airway and swallowing are often the functional problems encountered during the neonatal period. Case 1 had severe airway obstruction, and a mandibular distraction performed at the age of 3 years benefited him by improving his airway and feeding and permitted closure of the cleft palate. His neck pterygia involved shortening of all the tissue planes, however, releasing the skin and platysma resulted in a reasonable improvement in the neck movement. He is planned for a further stage of neck release. Any definitive surgery will be planned after the completion of his skeletal growth.

Case 2 has a mild form of PPS and has been managed throughout her developmental period. The bilateral CLP was managed according to the ACFU protocol until after the completion of her skeletal maturity. The lower limb pterygia was managed conservatively with complete functional recovery. At the other extreme, Case 3 represents a severe form of PPS with lower limb pterygia, multiple digital anomalies, and multiple facial and genital deformities.

The range of clinical features associated with multiple pterygium syndromes is peculiar. These highlight the benefit of multidisciplinary assessment and management to deal with multiple anomalies that are associated with this condition.

Due to the undetermined growth pattern of multiple pterygium syndromes (MPS of Escobar and PPS), it is suggested

that all such patients should be longitudinally followed up at least until maturity.

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MANAGEMENT OF FACIAL DYSMORPHO- GENESIS IN NEMALINE MYOPATHY: A CASE REPORT

Nemaline myopathy is a rare congenital muscle disease, which is clinically and genetically heterogeneous. Both neonatal and adult onset can occur, in those with neonatal onset, the resulting muscle weakness can also afflict the facial musculature and hence influence facial growth and development. This article reports on a case in which no orthodontic intervention was undertaken during childhood and adolescence. An early decision was made to treat the facial dysmorphogenesis surgically once skeletal maturity had been reached. The authors discuss and illustrate the untreated facial growth in this condition and the surgical outcome following orthodontic treatment and orthognathic surgery. World J Orthod 2005;6:xx-xx.

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A caucasian female, 10 years of age, was referred by a pediatrician to the craniofacial unit with a diagnosis of nemaline myopathy, for assessment of her associated facial dysmorphogenesis. She exhibited the characteristic dysmorphic long narrow face with hypotelorism (Fig 1). She had a severe anterior open bite with a Class II Division 1 malocclusion on a skeletal Class II base. The anterior open bite was such that she modified her dietary intake to avoid chewing food and she had an abnormal tongue thrust. This, along with articulation difficulties, resulted in difficulties with speech.

After multidisciplinary assessment, it was concluded that orthognathic surgery was inevitable in her case and so the decision was made not to intervene with orthodontic treatment at this

stage. In particular, functional appliances were thought to be inappropriate. Therefore, orthodontic treatment was postponed until preparation for orthognathic surgery commenced when she was skeletally mature. However, speech therapy was undertaken shortly after her initial referral, to treat the tongue thrust and the articulation difficulties.

When the patient was skeletally mature, at 16 years of age, a further comprehensive multidisciplinary planning meeting was undertaken. Her facial appearance is shown in Fig 2 and her abnormal dental arches are shown in Fig 3. In addition to her appearance, she was noted to have mild hypernasality and persistent articulation errors in her speech due to the narrow dental arch and the reduced muscle tone.



Fig 1 Patient at the time of referral, 10 years of age, with the characteristically dysmorphic "long face".



Fig 2 Patient at 16 years of age, once growth was complete.

TREATMENT PLAN

The treatment plan consisted of bimaxillary orthognathic surgery, preceded by decompensating orthodontic treatment. The surgical plan was for an impaction of the maxillary jaw 8 mm posteriorly, but no movement anteriorly, to allow autoro-

tation to shorten midface height. For the mandible, sagittal split osteotomy advancement by 5 mm was recommended to produce a Class I incisal relationship and a positive overbite and overjet. Secondly, and at a later date, rhinoplasty, genioplasty, and malar onlays were planned.

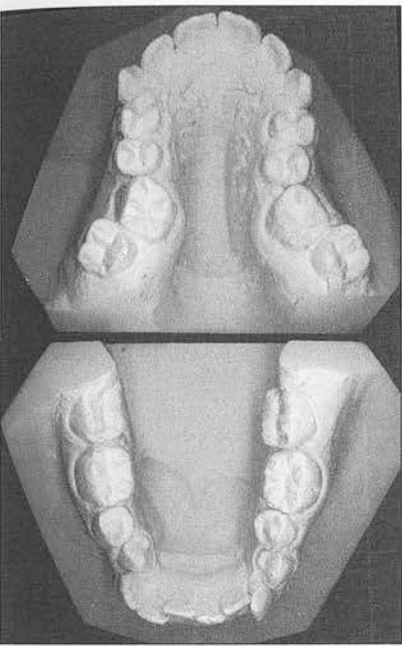


Fig 3 Dental casts at 16 years of age, prior to the rapid maxillary expansion and occlusal decompensation.



Fig 4 Patient at 18 years of age, following orthognathic surgery.



Orthodontic management prior to surgery consisted of expansion of the maxillary arch, without extractions, and alignment of the teeth. In addition, proclination of the maxillary incisors was undertaken to decrease the nasolabial angle, as well as flattening of the occlusal plane. In the mandibular arch, the teeth were aligned and the incisors were retroclined to increase the overjet.

Twelve months following the commencement of orthodontic treatment, orthognathic surgery was undertaken under general anesthesia. Orthognathic surgery consisted of the planned bimaxillary movements and onlay bone grafts to augment the hypoplastic malar bones. The patient made a good recovery from surgery and was discharged 2 days later. It was apparent postoperatively that she had deterioration in her speech articulation; however, this improved spontaneously over the next 3 months. The postoperative view is shown in Fig 4.

At the time of this writing, the patient is 18 months postoperative. The position and occlusion are stable and she is

delighted with her appearance. She will subsequently undergo reduction rhinoplasty and genioplasty.

DISCUSSION

Nemaline myopathy is a rare congenital muscle disease that is both clinically and genetically heterogeneous. The condition is unusual in that it can result both from autosomal-dominant and autosomal-recessive gene mutations.¹ The resulting condition is the phenotype arising from these different gene mutations. The definitive diagnosis of the condition is usually made on histologic examination of the muscle biopsy specimens, which demonstrate characteristic abnormal rod structures. However, it has recently been reported that there is no correlation between the degree of abnormality of the histologic specimen and the severity of the subsequent clinical course.²

The condition is also curious in that both neonatal and adult onset can occur. Those with neonatal onset are of particu-

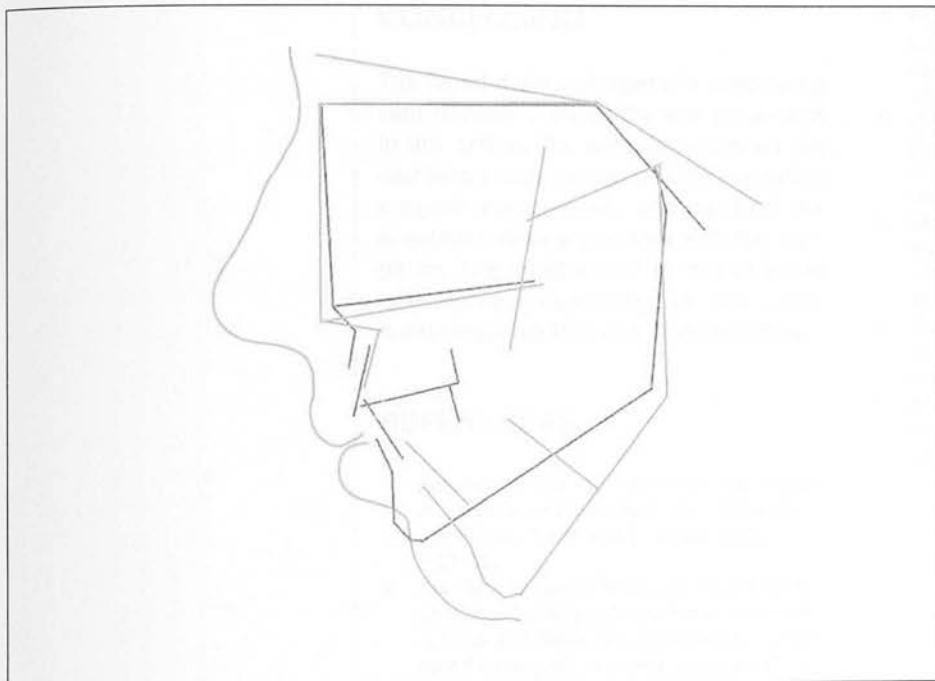


Fig 5 Outlines of the tracings of serial lateral cephalograms demonstrating the natural facial growth. The red line represents 12 years of age; the black line represents 16 years of age. Overall, the mandibular growth is marked, but there has been minimal anteroposterior growth of the maxilla.

lar interest because their resulting muscle weakness can also afflict the facial musculature, which may influence facial growth and development. The condition may produce a variety of clinical signs identifiable by clinicians. The characteristic "long face" is striking and has been previously reported in the literature.³ Quite apart from the long face, other signs include an associated speech disorder, and both dysphagia⁴ and ophthalmoplegia⁵ have been reported.

The treatment options for the facial appearance include orthodontics alone or combined with orthognathic surgery. However, there are a number of disadvantages to interceptive orthodontics. When considering orthodontic intervention, it must be remembered that the vectors of dysmorphic jaw growth differ from normal. In cases such as this one, where craniofacial growth is often unpredictable, an early decision regarding the likelihood of ultimate orthognathic correction is required before embarking on interceptive therapy. If orthodontic intervention were to be attempted, then the long-term skeletal effects of functional appliances on the abnormal musculature are questionable. Also, while vectors of

relapse may be predictable in nondysmorphic cases, relapse vectors in dysmorphic cases are uncertain. This reason, and the certainty that corrective surgery was inevitable in this case, led to the decision not to pursue interceptive treatment.

One result of this decision was that the natural growth pattern of this peculiar condition could be studied. Serial lateral cephalograms highlight that there has been marked mandibular growth but little development of the middle third of the face. These lateral cephalograms are shown in Fig 5.

If surgical intervention is contemplated, as in this case, there are also important considerations. Any procedure requiring general anesthesia needs to be undertaken with care, particularly in patients with nemaline myopathy. This is because there are particular risks associated with general anesthesia in patients with this condition, including aspiration, hypoventilation, and predisposition to malignant hyperthermia.⁶ The difficulties with anesthesia in this condition and their management have been summarized in the literature.^{6,7}

CONCLUSION

The facial dysmorphogenesis associated with nemaline myopathy was presented in this article. The authors described the combined orthodontic and orthognathic surgical management, and highlight the anesthetic risks associated with this condition. The longitudinal record of facial development unaffected by any orthodontic manipulation was demonstrated.

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An unusual complication of mandibular distraction

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Summary. *Background.* The authors present an unusual complication of mandibular distraction in a child with the curious condition of multiple pterygium syndrome is presented.

Case report. The patient was a Caucasian male with severe pterygia in his neck. As a result of his limited mouth opening and restricted upper airway leading to obstruction, he underwent lengthening of his mandible by distraction, which significantly improved his breathing. During his follow-up, it was observed that an unusually elongated permanent molar was present in an abnormal position.

Conclusion. This case highlights the need to carefully plan the sites for osteotomy and the potential for damage to the developing permanent dentition in young children.

Introduction

Multiple pterygium syndrome is comprised of severe neck contracture and other craniofacial anomalies. The associated mandibular hypoplasia may exacerbate the upper airway obstruction. Mandibular distraction is an established method to improve the upper airway in young children with airway obstruction caused by mandibular hypoplasias, such as Pierre Robin sequence, Treacher Collins syndrome and Nager syndrome [1–3]. Mandibular distraction has been shown to increase the minimum cross-sectional area of the upper airway that is maintained throughout the period of growth [1]. However, mandibular distraction can also be associated with complications including infection, haemorrhage, incomplete osteotomy, dislodgement of pins, failure of distraction, granuloma, abscess and inferior alveolar nerve damage, and requires great care in young children [4].

The authors report what they believe to be a previously unreported complication following mandibular distraction in a 3-year-old boy with multiple pterygium syndrome and Pierre Robin sequence. They present a long-term complication of distortion of a permanent tooth follicle.

Case report

A 3-year-old boy presented to the Australian Craniofacial Unit, Women's and Children's Hospital, North Adelaide, South Australia, Australia, with multiple pterygium syndrome, Pierre Robin Sequence and cleft secondary palate. The subject had severe contractures in his neck, axillae, elbows, wrists, groins, knees and ankles. Other facial findings included a scaphocephalic head, bilateral epicanthal folds, antimongoloid slant and malposition of the ears (Fig. 1). He had a hypoplastic lower jaw with his chin almost adherent to the sternum, and obstructive sleep apnoea as a result of a small nasopharyngeal passage.

Oral examination was difficult because of the limited mouth opening. The patient had previously required multiple dental extractions, which were carried out for dental caries that was a result of poor hygiene exacerbated by limited mouth opening. He underwent multidisciplinary assessment, including a review by a respiratory physician. A formal sleep study reported significant episodes of obstructive apnoea. Plain radiographs, a computed tomography scan, three-dimensional reconstruction and magnetic resonance imaging to assist in evaluating his underlying anatomical structures supplemented his investigations.

The subject subsequently underwent bilateral mandibular osteotomy, and distraction was performed at the rate of one millimetre per day. A mandibular lengthening of 12 mm was achieved (Figs 2 and 3). After stabilization for 3 weeks, the distractors were

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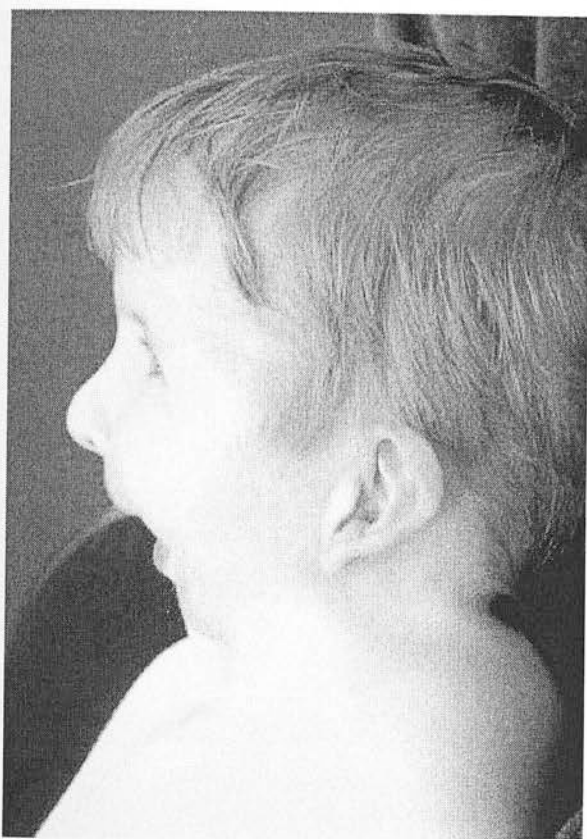


Fig. 1. Photograph of the subject showing severe pterygia of the neck and a hypoplastic mandible.

removed and his cleft palate was repaired at the same time. A subsequent sleep study indicated no further apnoeic episodes. The patient was also noted to have improved mouth opening and improved feeding. He has been kept under regular review, and his airway has remained patent.

However, an orthopantomogram at the age of 8 years revealed a curious elongated permanent molar lying in an abnormal position that was symptomless (Fig. 4). A review of the radiograph demonstrated that the first permanent molar was involved in the osteotomy site and was itself distracted. All radiology films were retrospectively reviewed, and it was noted that he had a well-developed crown of left lower molar prior to osteotomy, as shown in Fig. 5. Subsequent radiological examination revealed that the osteotomy site did not involve the crown of the molar on the left side (Fig. 6). His remaining dentition had unremarkable dental anatomy.

The subject's mouth opening deteriorated, and as a part of his subsequent management at the 11 years of age, a nylon model was constructed to plan his



Fig. 2. Photograph showing limited mouth opening.

surgery. This revealed grossly hypertrophied coronoid processes, a markedly hypoplastic mandible and a very obtuse body ramus angle (Fig. 7).

The patient subsequently underwent stage release of his neck contracture using skin graft, which permitted improvement of his mouth opening. He will require further release of his neck contracture and will be continuously reviewed by the multidisciplinary team according to the Craniofacial protocol throughout the growth period.

Discussion

Mandibular distraction is an established procedure to improve the upper airway in Pierre Robin sequence, Treacher Collins syndrome, Nager syndrome and other similar conditions with apnoeic attacks [1–3]. The presence of severe obstructive apnoea, which was identified in this patient by the respiratory physician, with pterygium syndrome directed the authors to lengthen the mandible by distraction instead of releasing the neck contracture as a first stage to facilitate mandibular growth potential.

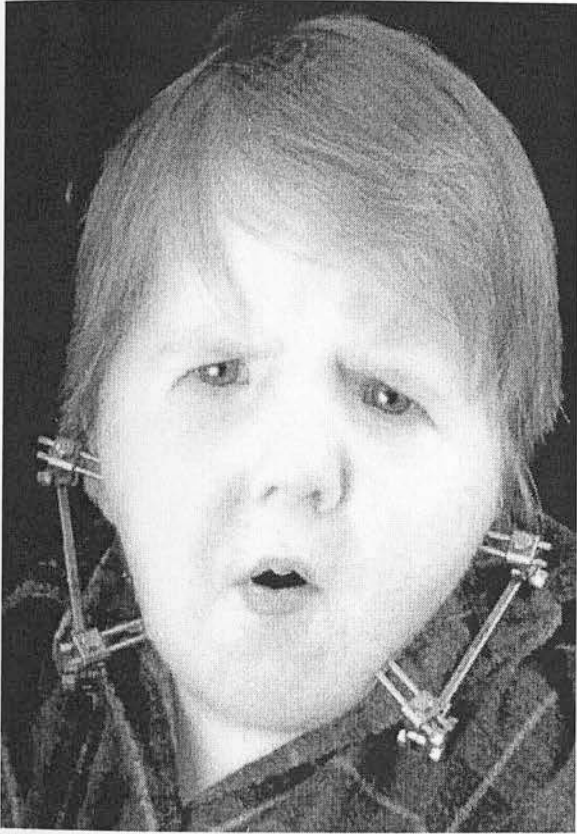


Fig. 3. Photograph showing linear mandibular distraction.

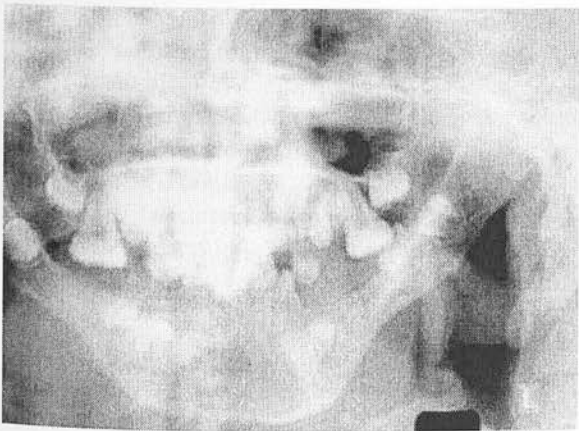


Fig. 4. Orthopantomograph showing the elongated permanent molar on the left side.

Multiple pterygium syndrome is a very rare congenital deformity with autosomal recessive inheritance manifested by multiple flexural contractures and secondary deformities involving the craniofacial region, spine, trunk, anogenital region and limbs [5]. The causes are probably heterogeneous, but decreased

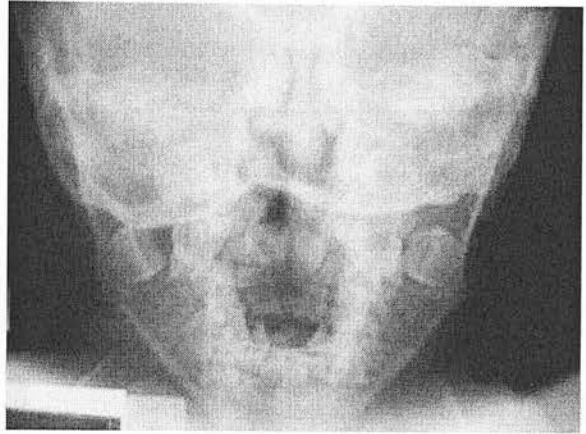


Fig. 5. Plain X-ray of the mandible prior to the osteotomy done when the subject was 3 years of age showing the well-developed crown of the left lower molar.

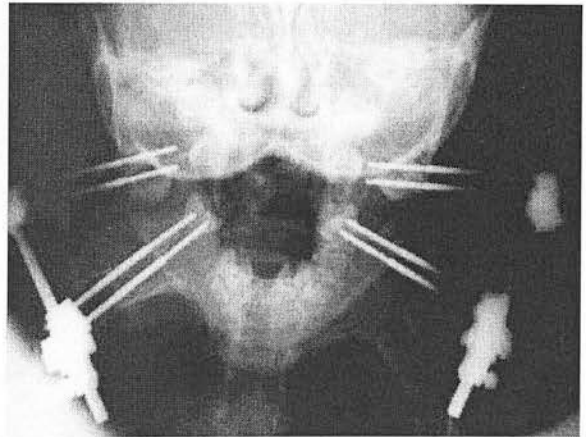


Fig. 6. Plain X-ray of the mandible after the osteotomy showing the intact lower molar on the left side.

foetal movement and neuromuscular pathology have been suggested [6].

Distraction of tissues has been utilized to recruit new bone and soft tissues following the principle of Ilizarov. Tissues including bone, muscle and tendon have been reported to undergo histogenesis during the distraction [7]. In this case, it would appear that the distraction force not only stretched these tissues, but may have elongated Hertwig's root sheath of the permanent molar as well. The dental development at the age of 3 years in his case was within normal limits. The other curious finding of the nylon model of this patient was the grossly hypertrophied coronoid process. It indicates that the temporalis muscle generated large forces over a prolonged period

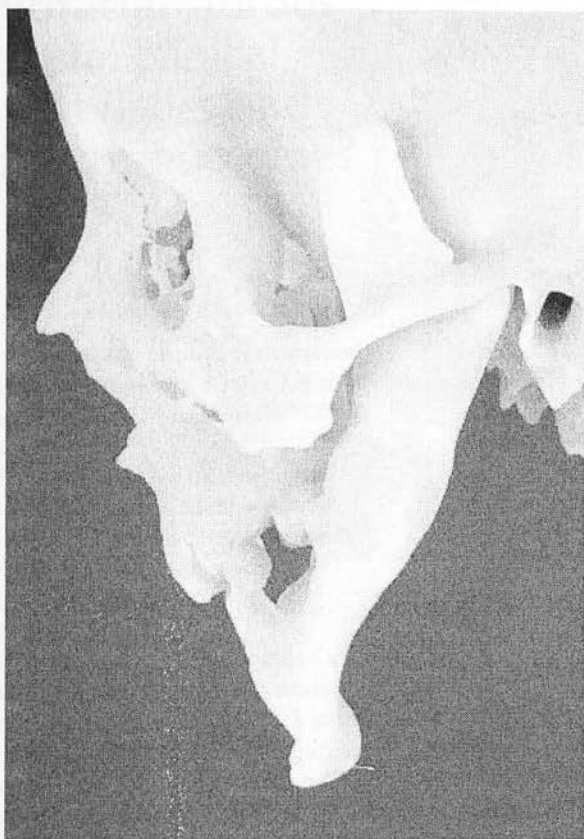


Fig. 7. Nylon model made when the subject was 11 years of age showing the hypertrophied coronoid process.

to achieve mouth opening, resulting in such an anatomical change (Fig. 5).

In summary, the authors report a late complication following mandibular distraction resulting in damage to the developing dentition, and it highlights the need for careful positioning of the osteotomy and the placement of pins. Careful review of dental radiographs should be undertaken to establish the position of the

dental follicle as part of the preoperative planning of the osteotomy site.

What this paper adds

- This paper presents a previously unreported long-term complication of mandibular distraction. Distraction as a part of airway management in those with mandibular hypoplasia may inevitably result in distortion of developing tooth buds.

Why this paper is important for paediatric dentists

- Paediatric dentists are an integral part of the multidisciplinary team that manages patients undergoing mandibular distraction. Mandibular distraction may inevitably alter the dental root morphology which has clinical implication should extraction be required.

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Craniofacial fibrous dysplasia: clinical characteristics and long-term outcomes

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CLINICAL STUDY

Abstract

Aim To present the clinical features and management outcomes in a large longitudinal series of patients with craniofacial fibrous dysplasia (CFD).

Methods Retrospective interventional consecutive case series. Main outcome measures included signs and symptoms, radiographic findings, long-term outcomes, and postoperative complications.

Results A total of 42 patients with CFD were identified. The mean age at presentation was 16.7 years; mean follow-up was 12.6 years. Out of these 42 patients, 37 (88.1%) had unilateral involvement and 5 (11.9%) had bilateral involvement, of which 3 (7.1%) had McCune–Albright syndrome. The commonest presenting symptom was facial asymmetry (36 cases, 86%). The frontal bone was the most commonly involved (27 cases, 64.3%), followed by the sphenoid (24 cases, 57.1%). The most common pattern of bone involvement was monostotic (32 cases, 76.2%). Radiological optic canal involvement occurred in 18 eyes of 15 (37.5%) patients, with optic atrophy in 9 eyes (18.8%) of 7 patients (16.7%). Surgical intervention was performed in 30 (71.4%) cases for both functional and reconstructive reasons. Optic canal decompression was performed in three cases, in all of which stabilization of vision was achieved; no patient lost vision as a result of surgery.

Conclusions In this large longitudinal series of CFD, visual loss was not uncommon and occurred insidiously. The presenting clinical and radiological features, surgical interventions, and outcomes are discussed.

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Keywords: fibrous dysplasia; optic neuropathy; optic canal decompression

Introduction

The first description of fibrous dysplasia is attributed to Von Recklinghausen in 1891,¹ although it was Lichtenstein who first coined the term ‘fibrous dysplasia’.² It is an uncommon, histopathologically benign disease, characterized by the replacement of normal marrow by proliferating fibro-osseous tissue, which expands and thins overlying cortex.³ Its aetiology is a post-zygotic, somatic mutation of the *GNAS* gene, which encodes the α subunit of the ubiquitous stimulatory G protein.^{4,5} Fibrous dysplasia is a non-familial disease with onset usually occurring by 10 years of age, although progression is known to occur into adulthood with associated cosmetic and functional morbidity.

Fibrous dysplasia occurs in a polyostotic form in 30% of cases or, more commonly, a monostotic form (70%).⁶ When limited to the craniofacial region, most authors consider the disease process to be monostotic even if several different bones are affected, as there is only one disease focus.⁷ Craniofacial involvement occurs in 50% of patients with polyostotic and 27% of patients with monostotic fibrous dysplasia.⁸ The McCune–Albright syndrome is a subtype of the polyostotic form associated with areas of cutaneous pigmentation and precocious puberty, first described by Albright and associates in 1937.³

Previous reports in the literature on ophthalmic manifestations include case series and numerous descriptive, often cross-sectional studies focusing on the question of prophylactic vs therapeutic optic nerve decompression.^{9,10} We present an analysis of a longitudinal series of 42 cases of craniofacial fibrous dysplasia (CFD), comparing their clinical features, radiological patterns, and long-term outcomes, with a mean follow-up period of 12.6 years.

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Materials and methods

A retrospective descriptive analysis of the clinical presentation, management, and postoperative course of 42 consecutive patients with radiologically confirmed fibrous dysplasia was conducted. Cases were identified from the Australian Craniofacial Unit database. These patients were managed at the Women's and Children's and the Royal Adelaide Hospitals in South Australia between 1975 and 2006. Ethical approval was obtained from the local institutional review board.

The diagnosis of CFD was established after clinical review by the craniofacial surgeons; all patients had radiological evaluation. A combined team consisting of an ophthalmologist and a craniofacial surgeon evaluated all patients. Histological confirmation of fibrous dysplasia was performed only if operative intervention occurred.

Data from ophthalmology reviews were obtained from the relevant case notes and were documented on standardized pro forma sheets. Exclusion criteria were: (1) previous craniofacial, cranial, or strabismus surgery; and (2) lack of preoperative ophthalmic assessment. The types of data collected are shown in Table 1.

Visual acuity was measured by standardized methods appropriate to age, with patients being considered to have impaired vision (in this study) if they had a visual acuity of 6/12 or less, or more than two Snellen lines poorer than age-adjusted normative values in at least one eye.¹¹ Visual fields were assessed by the Humphrey

Visual Field/Swedish Interactive Thresholding Algorithm 30-2 computerized testing (Allergan-Humphrey), or Goldmann perimetry testing. Optic nerve dysfunction was defined as the presence of a characteristic scotoma (or field deficit), or an abnormal result on two of four other tests: visual acuity worse than 6/12, correct identification of fewer than 10 of the 14 Ishihara colour plates, the presence of a relative afferent pupillary defect, or evidence of optic nerve head pallor on fundal examination.

Axial and coronal computerized tomographic (CT) sections were obtained. Where possible, high-resolution images of the orbit were performed using a 0.5 mm helical run from the upper teeth to the top of the frontal sinuses in a 200 mm field of view. Three-dimensional reconstruction was performed on the optic canals that were suspected to be narrow on initial CT scanning and sections perpendicular to the longitudinal axis of the optic canal were generated. The dimensions of the optic waist were measured with digital callipers on the reformatted images and the area of the optic canal was calculated using the method described by Lee *et al*¹² (half the height in mm \times half the width in mm $\times \pi$, where $\pi = 3.14$).

Surgery was undertaken for functional or reconstructive reasons largely based on criteria outlined in the surgical classification described by Chen and Noordhof¹³ (Table 2).

All patients were followed up by clinical examination, plain radiographs, and CT scanning, ranging from 2 months to 31 years post-diagnosis (mean 12.6 years). The frequency of follow-up was tailored according to disease aggressiveness, the presence of optic nerve compromise, and the type of intervention performed.

Results

Demographics

A total of 42 patients were identified. The mean age at presentation was 16.7 years (median 15 years; range 0–59 years). There were 20 women (47.6 %) and 22 men (52.4%). Of 42 patients, 36 (85.7%) were Caucasian and 6

Table 1 Types of data collected

Demographics

Patient age
Gender (52% male, 48% female)

Major symptoms/signs

Facial asymmetry (86%)
Mass (64%)
Epiphora (7%)
Headache (20%)
Blurred vision (24%)
Eyelid position abnormalities (10%)
Loss of visual field (8%)
Diplopia (8%)
Sinusitis (8%)
Epistaxis (3%)
Hearing loss (3%)

Major signs

Visual acuity
Relative afferent pupillary defect
Colour vision with the use of 14 Ishihara colour plates
Examination of the fundus
Ocular motility dysfunction
Measurement of proptosis with a Hertel exophthalmometer
Measurement of interpupillary and intercanthal distances

The percentages in brackets represent the frequency of each entry in this series.

Table 2 Surgical classification of craniofacial fibrous dysplasia

Zone 1	Frontal, orbital, nasal, ethmoid, zygoma, upper maxilla	Surgical treatment for epiphora, extraocular motility disturbance, proptosis
Zone 2	Parietal part of the occipital, temporal, (lateral cranial base)	Surgical treatment largely instituted for cosmetic reasons
Zone 3	Central cranial base petrous, mastoid, pterygoid, sphenoid	Surgery avoided until the appearance of symptoms
Zone 4	Maxillary alveolar bone, mandible	Teeth-bearing bones, conservative treatment

Adapted from Chen and Noordhoff.¹³

(14.3%) were Asian. The mean duration from the first signs or symptoms to the diagnosis of fibrous dysplasia was 5.6 years (median 5 years; range 0–40 years). Thirteen (31.0%) patients were diagnosed after the age of 18 years.

Thirty-seven (88.1%) cases had unilateral craniofacial involvement and five (11.9%) had bilateral involvement, of which three (7.1%) presented with the McCune–Albright syndrome.

Clinical presentation

Data on the presenting signs and symptoms of patients are summarized in Table 1. The most common presentation was facial asymmetry, which occurred in 36 (86%) patients, followed by an orbital or facial mass 25 (60%). The most common ocular presentation was blurred vision, which occurred in 10 (24%) patients, followed by eyelid position abnormalities that occurred in 4 (10%) patients.

Vision

Visual acuity was initially worse than 6/12 in 9 (18.8%) of the 48 examined eyes, due to compressive optic neuropathy in 6 (12.5%) eyes and amblyopia in 3 (6.3%). Three further eyes were diagnosed with optic neuropathy (total nine eyes in seven patients), in two of whom the visual acuity had declined to less than 6/12 by the end of follow-up; in the third patient, visual acuity remained stable. In those eyes with optic neuropathy, the median final visual acuity was counting fingers (range 6/9 to perception of light). In this series, no patient presented with acute loss of vision, with visual loss developing insidiously in all cases. Surgical optic canal decompressions were performed in three cases, one of which was prophylactic and two of which were performed for clinical signs of optic nerve compromise; in these patients, the visual acuity was successfully prevented from declining postoperatively, with follow-up of 3–23 years (mean 11.2 years).

Globe displacement

Proptosis was present in 20 (42.6%) eyes; the mean axial displacement was 2.9 mm (median 2.5 mm, range 1–8 mm). Globe displacement occurred in 34 (72.3%) eyes and the mean non-axial displacement (ie vertical or horizontal dystopia of the globe) was 2 mm (median 2 mm, range 1.5–10 mm).

Radiological findings

The type of radiological bone involvement on the CT scans included: sclerotic in 21 (50%) patients, pagetoid in

11 (26.2%) and cystic in 2 (4.8%) (no mention in archived radiological report, 8 cases).

The most common reported pattern of bone involvement as determined by CT was monostotic (Figure 1), observed in 32 (76.2%) patients, compared to polyostotic disease, observed in 10 (23.8%) patients (Figure 2). Of those with monostotic disease, involvement of multiple bones across suture lines occurred in 22 (68.8%) cases and single bone involvement



Figure 1 Contrast-enhanced axial computed tomography (CT) scan (bone windows) showing a monostotic variant of craniofacial fibrous dysplasia, centred on the right ethmoid bone and obliterating the right posterior ethmoid sinus cavities.

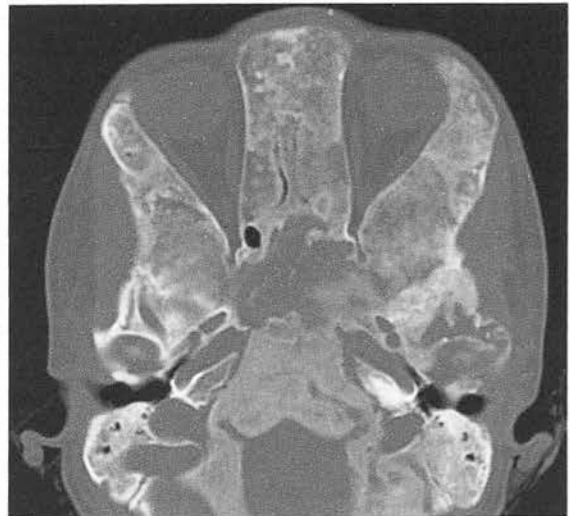


Figure 2 Axial CT scan (bone windows) showing a polyostotic variant of craniofacial fibrous dysplasia, associated with the McCune–Albright syndrome. The bones are grossly expanded with thinning of the overlying cortex. Note the presence of both cyst-like areas as well as more homogenous areas with a so-called 'ground-glass' appearance.

occurred in 5 (15.6%) (no mention in archived radiological report, 5 cases). Radiological sinus involvement occurred in 22 (52.4%) patients. The frontal sinus was the commonest to be involved (14 (63.6%) patients), followed by the ethmoid (12 (54.5%)), the sphenoid (6 (27.2%)), and the maxillary sinus (5 (22.7%)). Single sinus involvement was the most common pattern occurring in 13 patients, with multiple sinus involvement in 9.

The orbital roof (frontal bone) was the most common site of orbital involvement, occurring in 27 (64.3%) patients, followed by the lateral orbital wall (sphenoid) in 24 (57.1%), with involvement of the floor (maxilla) and medial wall (ethmoid) occurring in 18 (42.9%) patients each.

Radiological involvement of the optic canal was seen in 15 (35.7%) patients, 3 of which had bilateral disease; of these, optic atrophy developed in 9 eyes (18.8% of all eyes affected, 50% of all cases of radiologically involved canals). Of these 18 optic canals, measurements of the optic canal had been performed for 10 canals (inappropriate CT images for performing measurements in other cases), 5 of which had optic neuropathy and 5 did not. On CT scans showing radiological optic canal involvement, the average optic canal waist height and width at the narrowest point was 3.70×3.26 mm (area 9.47 mm^2) in the group with no optic neuropathy, compared with 3.52×3.20 mm (area 8.84 mm^2) in the group with optic neuropathy.

Histological findings

Histological confirmation of the disease was performed in all cases that underwent surgery (30 cases, 71.4%; Figure 3). No cases showed malignant transformation over the period of observation.

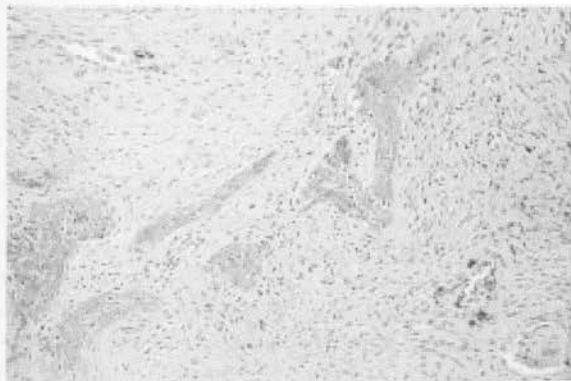


Figure 3 The typical histology of fibrous dysplasia is shown here from one of the cases that underwent surgical intervention. Curvilinear bony trabeculae with irregular margins are contained within a cellular fibrous background.

Surgical intervention and outcomes

Operative intervention was performed in 30 (71.4%) cases; the median time to surgical intervention was 1 year from diagnosis. Radical resections of orbital bones in various combinations were performed in 23 cases; bone contouring was performed in 4 cases. Surgical optic canal decompression was performed in three cases, one of which was prophylactic. Of all those patients that had surgery, 11 required more than one procedure over the follow-up period.

Of the 18 radiologically involved optic canals, three underwent optic canal decompression. In the two therapeutic decompressions performed for compressive optic neuropathy (both in monostotic disease), the visual acuity did not decline further postoperatively (stable at 6/18 in one case, 6/36 in another). In the third case, a prophylactic decompression was performed in a 13-year-old girl with polyostotic disease; the preoperative acuity was 6/6 and there was no subsequent deterioration or progression of disease. The mean follow-up period for these three patients was 11.2 years (range 3–23 years). Of the 15 involved canals that were not decompressed, optic neuropathy developed in 7 eyes and of these 7, the visual acuity deteriorated in only 2 eyes (6/9 to 6/18 and 6/9 to hand movements); a relative afferent pupillary defect or optic atrophy developed in the remaining 5 patients. In the two patients in whom visual decline was noted, there were no significant differentiating features in their respective patterns of disease or demographics.

Postoperative complications occurred in 15 (50%) patients, including infection, binocular diplopia, and cranial nerve palsies in 3 (10%) patients each; pain in 2 (13.3%); epistaxis, hypertrophic scar, anaemia, and ectropion in 1 (6.7%) each.

Follow-up

The mean follow-up for the population was 12.6 years (range 2 months to 31 years). Over the period of observation, radiological progression of craniofacial bones other than the optic canal occurred in three cases, but clinical deterioration of compressive optic neuropathy occurred in two cases.

Discussion

Craniofacial fibrous dysplasia is a histologically benign disease^{13,14}; however, our series at a tertiary referral centre demonstrates that its presentation usually occurs early in life and a degree of visual loss is not an uncommon complication (18.8% at presentation in this series). The majority of patients required surgical intervention during the course of the disease, mainly

due to facial asymmetry and orbital mass effect. It should, however, be noted that our experience likely reflects the tertiary referral of more severely affected cases.

Disease presentation

Fibrous dysplasia has traditionally been regarded as a disease of childhood, with symptoms developing most often within the first two decades of life. The dysplastic expansion becomes quiescent after patients reach puberty in 60–80% of cases; however, the end point of tumour growth is unpredictable and enlargement has been observed through the seventh decade.¹⁴ In our studied population, the mean age at presentation was 16.7 years. Of the 30 patients that underwent surgical intervention, 19 (63.3%) were treated surgically before the age of 18 years (the period of most rapid dysplastic expansion); however, the remaining 11 (36.7%) patients treated surgically were 18 years and older.

The typical picture of CFD depends on the expansion and compression of pathological bone against adjacent structures.⁶ In the largest reported series of 66 patients, Becelli *et al*¹⁵ found that in 24 (36.3%), the disease was lacking evident symptomatology. In a further series of 27 cases, Yavuzer *et al*¹⁶ found that painless bony enlargement resulting in skeletal deformity and gradual asymmetry was the most common symptom. In our studied population, 36 (76.6%) patients presented with facial asymmetry, although 19% had headache at presentation, considerably less than 7/10 (70%) of cases noted to have ipsilateral headache or orbital pain by Rootman.¹⁷

Radiological pattern of involvement

The radiological pattern of bone involvement in this series revealed a predominance of sclerosis; this has been variable among the different published series.^{10,18,19,20,22} Rootman *et al*¹⁸ have found the pattern to be equally distributed among the two groups, sclerotic and pagetoid. Others have found pagetoid to be the most common.¹⁰ In our series, most patients had monostotic lesions that involved multiple adjacent bones and crossed suture lines, which is in agreement with that reported in other series.¹⁹ However, distinction between monostotic and polyostotic forms can be difficult because of the intimate connection of the individual craniofacial bones.²⁰

In two major series, the frontal bone was involved in 58% of cases and the sphenoid in 35%.⁸ Other reports have found the maxilla to be the commonest bone involved.¹⁰ As in our series, Rootman *et al*¹⁸ found that

the frontal bone was the commonest involved bone in the orbital region.

Visual loss and optic nerve decompression

Visual disturbance has been reported to occur in up to 18% of cases due to optic canal involvement,⁸ a figure similar to the prevalence in our series, in which compressive optic neuropathy occurred in nine (18.8%) eyes. Chronic visual loss due to compressive optic neuropathy was the only pattern of presentation of visual loss in our series; this contrasts with other series that have reported a predominance of acute visual loss in fibrous dysplasia.¹⁰ In addition, amblyopia was common in our series, leading to a visual acuity of <6/12 in three eyes (6.3%), a figure considerably higher than the normal 2.9%, according to population data for children between 4 and 10 years.²¹ This high prevalence of amblyopia is hard to explain but may relate to the high degree of facial deformity in our patients.

Optic canal decompression surgery can be either therapeutic or prophylactic, with some surgeons performing contralateral prophylactic surgery at the time of initial therapeutic decompression.⁹ Despite the optic nerve's putative lack of plasticity, improvement following therapeutic surgery is well documented.^{9,10} Nevertheless, the realistic goal of such surgery is to maintain vision and must be balanced against the risk of postoperative visual loss.

The case for prophylactic decompression of the optic canal revolves around the unpredictability of onset of acute visual loss (often due to the development of an aneurysmal bone cyst or mucocoele) and the short amount of time before it becomes permanent,⁹ although treatment with corticosteroids and urgent surgical decompression may occasionally reverse acute visual loss.¹⁰ However, the function of prophylactic surgical intervention is controversial as resection of dysplastic bone carries a risk of surgically induced visual loss.¹ Proponents of prophylactic surgery argue that optic canal involvement by fibrous dysplasia heralds visual dysfunction: Chen *et al*⁹ found that of 18 patients with clinical or radiological evidence of optic canal involvement, 12 (67%) had some degree of visual loss, with 6 of these 12 having visual acuities of hand motion or less, 1 of whom was bilaterally affected. A recent study by Lee *et al*¹² challenged these findings, showing that encasement of the optic canal in fibrous dysplasia was not correlated with visual loss in 38 patients. However, this was a cross-sectional study, conducted at a point in time, and can also be criticized for selection bias: patients who had previously undergone therapeutic optic nerve decompression (ie those patients with visual loss) were excluded from the study. In another recent

cross-sectional case series, Cruz *et al*⁷ also found that radiological evidence of apical involvement in fibrous dysplasia (55.9% of apices were narrowed, defined as circumferential narrowing) does not necessarily cause a clinical optic neuropathy; however, it is not clear whether patients, who might have already sustained visual loss, were included in the study. In summary, it is clear that optic canal narrowing *per se* does not necessarily cause optic neuropathy; from the available data, however, in the absence of aneurysmal bone cysts or mucocoele formation, it is not yet clear whether prophylactic decompression of the optic nerve in fibrous dysplasia is indicated or not.

The relatively high incidence of chronic visual loss as a result of compressive optic neuropathy in our longitudinal case series would seem to support early intervention. However, the majority of our patients with radiological stenoses did not progress over the observation period and in those seven eyes with radiological optic canal involvement and compressive optic neuropathy, who did not undergo surgery, the visual acuity did not deteriorate in four (over a mean follow-up of 19 years). Although only a randomized clinical trial will provide a definitive answer, our current practice is not to perform prophylactic decompression, but to monitor those with radiological involvement of the optic canal.

Other surgery

Surgical treatments can be broadly categorized into two different techniques: a conservative approach, consisting of the shaping of the dysplastic bone tissue, often repeated over time, avoiding the removal of bone (or postponing it until it becomes indispensable); and a radical approach, consisting of removal of the pathological bone tissue and its reconstruction with an autologous bone graft.²² Surgical interventions were performed on 30 (71.4%) patients in our series, with 11 requiring further surgery over the course of follow-up.

Lesions that are expected to demonstrate aggressive behaviour include those associated with excessive hormonal drive (McCune-Albright syndrome) and those associated with growth hormone-producing tumours.⁹ Of the three cases of McCune-Albright syndrome in our series, a 6-year-old male child did not require operative intervention over 19 years of follow-up, another 6-year-old male child required a transcranial resection at 2 years after diagnosis, and an infant required three debulking procedures over 7 years of observation. All three cases had a sclerotic radiological appearance on CT, which is consistent with an aggressive nature of disease with an earlier onset.

Malignant transformation

Malignant transformation of fibrous dysplasia occurs infrequently, with reported frequencies ranging from 0.4 to 4%.²³ None of our cases demonstrated malignant change over the period of observation.

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CHAPTER SIX

INTRODUCTION

Scientific research is essential if our understanding of the altered craniofacial morphology and anatomy due to the underlying pathological processes are to be improved. The development of new technologies including three dimensional CT scanning allows more accurate definition of changes in morphology which occur in different disease processes affecting the craniofacial region. With improved understanding in morphological changes the differences from normal anatomical structures enables a refining of management and the surgical techniques used for correction.

Additional knowledge provided by molecular biology and the study of disease processes at a cellular level is an opportunity which provides the possibility for developing new therapeutic regimens which could alter the natural history of the disease process. This is a significant goal in the management of craniosynostosis where treatment has remained surgically based for over a hundred years.

In this chapter the first group of papers are studies using morphometric analysis of cranial disease and how the results of detailed studies can affect clinical management. The second group of papers are as a result of collaborative work with my colleague A/Prof Barry Powell, a molecular biologist. We have jointly directed the work of the

Craniosynostosis laboratories studying molecular changes affecting cranial suture cells since 2005. This longitudinal investigation into the fundamental cause of disease is supported by the NHMRC in Australia. It has the ultimate aim of trying to develop non-surgical adjuvant therapeutic treatments for children with severe phenotypes of craniosynostosis where currently the only treatments are multiple transcranial surgical interventions, which may need to be repeated during childhood.

CHAPTER SIX - PAPERS

The first paper is a morphometric analysis of children with plagiocephaly resulting in asymmetrical head shapes. The causes can either be positional plagiocephaly or may result from a unilateral synostosis affecting either the lambdoid or the coronal sutures. This investigation undertakes detailed shape analysis and compares the results for the different underlying causes and identifies a number of landmarks and measurements which can be used to distinguish between the different groups.

The second paper is an investigation into cervical spine anomalies using 3D CT scans of children with cleft lip and palate. Previous investigations had used two dimensional conventional radiography, often with cephalograms which only demonstrated the upper cervical spine. This study identified a range of anomalies, including smaller vertebral bodies suggesting growth disturbance in the cervical spine development may occur in association with formation of cleft lip and palate deformity.

The third paper is an investigation into the hyoid bone in cleft lip and palate children, again using 3D CT scans. This identified a range of anomalies and suggested that the growth and development of the hyoid bone may also be abnormal in cleft lip and palate children.

The fourth paper was an investigation into the asymmetry of the nasal bones in children with unilateral cleft lip and palate, when compared to isolated cleft palate and bilateral cleft palate. It found that the growth of the nasal bones appears to be affected by the cleft, even though it is some distance from the cleft itself and results in nasal bone asymmetry.

The fifth paper is a study into the intracranial volumes in children with non-syndromic craniosynostosis. This has been studied previously and measurements made either directly with water or seeds or indirectly using two dimensional radiology. This study using refined measuring techniques and 3D CT scanning technology to try to precisely measure the volume. The findings are very important for surgeons since it follows that the goal of surgical treatment for affected children should be calvarial remodelling rather than volume expansion.

The sixth paper was a review of the morphological and histological changes associated with the primary and secondary dentitions in Apert syndrome children. It identified morphological anomalies of the enamel and dentine not previously recorded. Combined with the morphological changes in crown and root morphology. This knowledge is important to paedodontists and orthodontists managing children who require good dental care for orthodontics because at skeletal maturity orthognathic surgery is inevitable, and relapse is prevented, at least in part, by pre-surgical orthodontic treatment.

The seventh paper is the first molecular biology study. In it microarray technology and a novel “*in vivo-in vitro*” approach to identify genes which are important in calvarial tissue differentiation have been undertaken as part of a study to improve understanding of the molecular processes in craniosynostosis. This collaborative study was undertaken using “state of the art” technologies available at Queensland University of Technology but directed by the Craniosynostosis laboratory team using tissue samples collected by myself from patients in Adelaide. This study identified genes which were known to be important in signalling pathways which had not been previously well recognised as having roles in craniosynostosis.

The eighth paper is a particularly significant paper which investigated changes in gene expression in sutural tissues, comparing fused and unfused cranial sutures of children with Apert syndrome and non-syndromic craniosynostosis, using microarray technology. This identified a number of novel genes to be differentially expressed as well as confirming differential expression in genes previously identified. The significance of the study is that it was the first time that gene changes had been found to be similar in both syndromic and non-syndromic cranial sutures. The implication is that studying human tissue from the more common non-syndromic craniosynostosis the results can be reasonably extrapolated to include rarer syndromic forms where

ultimately a medical therapeutic intervention (possibly developed from differential gene expression) is most desirable.

The ninth and final paper a molecular biological study to attempt to manipulate culture media to reproduce the gene expression in cranial suture cells which is lost on culture of the cells when they become de-differentiated. The outcome was that although changes in cells markers do occur in different osteogenic media, no conditions were able to recreate the same genetic expression as the human cranial tissues. This final paper highlights the need for caution in interpreting results from cell culture studies of cranial suture cells and emphasizes why it éssential that scientific investigation in this topic is rigorous, even though the price of care is that true progress may be slow .

In summary the morphology papers studying cleft lip and palate craniofacial morphology demonstrate that morphological changes can occur much further away from the site of the cleft than had previously been recognised. The molecular studies to date raise the possibility that adjuvant non-surgical treatment remains a possibility in the future. However, much remains to be done. Further functional studies of the newly identified differentially genes in human cells and tissues as well as animal studies are required to select possible treatments before human trials could be considered.

Three-Dimensional Computed Tomography Cephalometry of Plagiocephaly: Asymmetry and Shape Analysis

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Objective: To investigate facial asymmetry associated with both deformational and synostotic plagiocephaly and to identify variables based on skeletal landmarks that distinguish the conditions and quantify severity.

Design: Retrospective, cross sectional.

Setting: Australian Craniofacial Unit, Adelaide.

Main Outcome Measures: Proportional differences between bilateral distances and principal component (PC) analysis of the skeletal landmarks.

Patients: The three-dimensional positions of 78 osseous landmarks were determined from computed tomography (CT) scans of 21 patients with deformational plagiocephaly (DP), 20 patients with unilateral coronal synostosis (UCS), and 2 patients with unilateral lambdoid synostosis (ULS).

Results: For both DP and UCS, significant asymmetry was found for the orbital depths, mandibular lengths, maxillary depths, zygomatic arch lengths, lateral base of the parietal bone, and the angle between the anterior and the posterior cranial base projected onto the axial plane. The small sample size for ULS precluded definitive statistical statements but allowed some useful comparisons with the other conditions. The first three PC scores were able to distinguish among the three conditions and which side was affected.

Conclusions: The asymmetry of the cranial base and facial structures, arising from localized abnormality or deformational forces in either the frontal or the occipital regions, can be quantified by a plethora of bilateral features or summarized by PC analysis.

KEY WORDS: asymmetry, coronal synostosis, deformational plagiocephaly, geometric morphometrics, lambdoid synostosis, plagiocephaly, principal component analysis, Procrustes

Plagiocephaly is a descriptive term that refers to an asymmetric, twisted head shape but does not indicate the underlying etiology of the morphological phenotype. Potential causes in-

clude deformational forces and craniosynostosis (Clarren, 1981; Hansen and Mulliken, 1994; Cohen, 2000). Examples of three forms of plagiocephaly are shown in Figure 1.

Deformational plagiocephaly (DP), also referred to as positional plagiocephaly, is the most frequently encountered form of plagiocephaly. Underlying causes include early engagement of the pelvis *in utero*, head binding, torticollis, myoneural dysfunction, and sleep position (Clarren, 1981). Subsequent preferential positioning of the child in the supine position poten-

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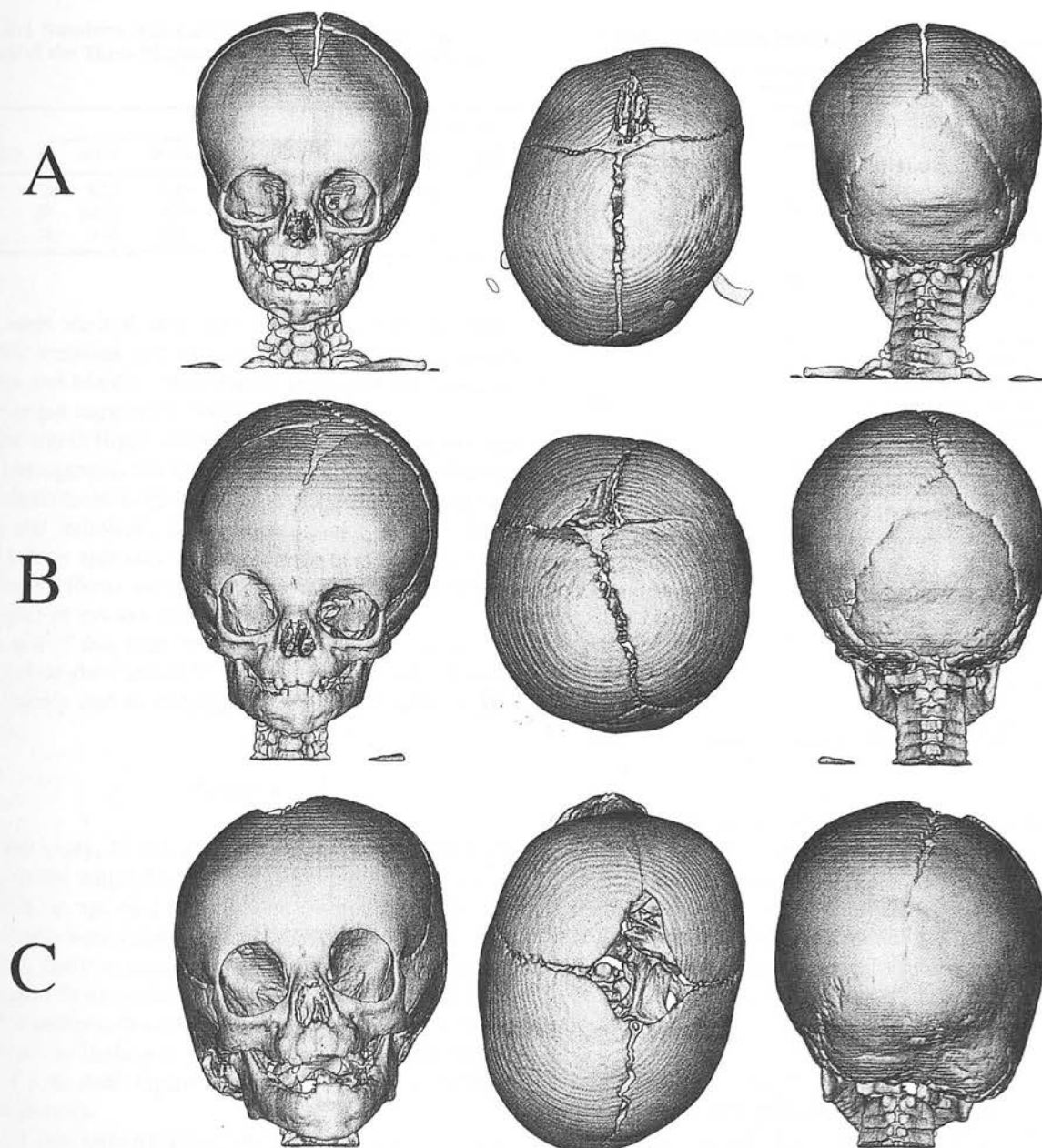


FIGURE 1 3D CT reconstructions of patients with plagioccephaly having distinct etiology. A: Right posterior DP. B: Right UCS. C: Right ULS.

tially increases the initial deformation, given the softness and pliability of the infant skull.

Unilateral craniosynostosis of a paired suture may also result in plagioccephaly. Unilateral coronal synostosis (UCS) refers to the premature fusion of the coronal suture on one side and occurs with an incidence of 1 in 10,000 live births (Cohen, 2000). Unilateral lambdoid synostosis (ULS) is significantly more rare, with an incidence of approximately 1% of those presenting with posterior DP (Menard and David, 1998). Craniosynostosis of metopic and sagittal sutures results in the distinctive but symmetric head shapes trigonocephaly and scaphocephaly, respectively.

Deformational plagioccephaly and unilateral synostosis have differing etiologies that require totally different approaches to

management. Patients with craniosynostosis often undergo surgical excision of the affected cranial sutures and cranial vault reshaping, whereas the management of patients with DP tends to be nonsurgical (David, 1992; David and Menard, 2000).

Each form of plagioccephaly can usually be distinguished through physical examination and radiology of the calvaria by an experienced clinician, although there have been recent "epidemics" of misdiagnosed ULS (Turk et al., 1996; Cohen, 2000).

Previous studies have quantified some of the skeletal craniofacial asymmetries that occur in plagioccephaly, such as orbital (Lo et al., 1996a), mandibular (Kane et al., 1996), and angular deviation of the anterior and posterior cranial base (Lo et al., 1996b). The present study has extended this work, using

TABLE 1 Numbers, Age Range, and Affected Side of the Patients of the Three Plagiocephaly Phenotypes: DP, UCS, and ULS

Diagnosis	n	Age (mo)				Ipsilateral	
		Mean	Median	Minimum	Maximum	Left	Right
DP	21	6.75	5.22	2.89	20.73	10	11
UCS	20	10.58	4.39	1.71	69.22	12	8
ULS	2	6.89	6.89	6.18	7.59	0	2

many more skeletal landmarks for determination of anthropometric variables and applying a shape analysis procedure (Dryden and Mardia, 1998) known as generalized Procrustes and principal component analysis (GPA/PCA).

Milne and O'Higgins (2002) have used GPA/PCA to investigate phylogenetic, functional, climatic, and scaling influences on the craniofacial morphology of macropods (kangaroos, wallaroos, and wallabies). Zollkofer and Ponce de Leon (2002) have used the approach to study patterns of craniofacial shape variation in *Homo sapiens* by using computed tomography (CT) scans of modern and fossil skull specimens.

The aim of this study was to apply GPA/PCA to quantify the extent of skull deformity in deformational and synostotic plagiocephaly and to distinguish among their different phenotypes.

METHODS

For this study, 21 patients with DP, 20 patients with UCS, and 2 patients with ULS (Table 1) were selected. One patient with UCS, at age 69.2 months, was substantially older than the rest, who were younger than 27 months. Because the morphometric analysis essentially removes size as a variable, it was decided to retain the data associated with this older child. For these patients, three-dimensional (3D) landmark positions of 78 osseous landmarks (Tables 2 and 3) were determined from CT scan data. Figure 2 shows the location of some of these landmarks.

The CT data were retrieved from the Australian Craniofacial Unit archives. Before 1993, a GE8800 CT scanner (General Electric Co., Fairfield, CT) was used with an acquisition protocol consisting mainly of 3-mm thick slices at 1.5-mm spacing through the face and of 5-mm thick slices at 3-mm spacing above the glabella with pixel size 0.75. During the years 1994 to 1999, patients were scanned with a GE-Advantage spiral CT scanner with 3-mm thick slices reconstructed every 1.5 mm and pixel size 0.488. From the year 2000, a GE multislice CT scanner has been used with axial slices reconstructed from spiral acquisition with spacing of 0.6 mm, thickness of 1.25 mm, and pixel size 0.488.

Landmark locations were determined from CT scan data by our own software, Persona (Netherway et al., 1995). The raw images were clipped and edited to remove unnecessary features such as endotracheal tubes and head restraints. Several windows were displayed simultaneously containing axial slices, reformats, and 3D CT reconstructions. A movable marker,

TABLE 2 Description of Midline Osseous Landmarks

Landmark	Abbreviation	Definition
Anterior nasal spine	ans	The apex of the anterior nasal spine
Basion	ba	The midsagittal point on the anterior margin of the foramen magnum (at the saddle point)
Bregma	br	The intersection of the sagittal and the coronal sutures on the surface of the cranial vault
Gnathion	gn	The most inferior point on the mandibular symphysis in the midsagittal plane
Infradentale	id	The most anterosuperior point on the mandibular alveolar margin in the midsagittal plane
Lambda	l	The intersection between the lambdoid and the sagittal sutures on the surface of the cranial vault
Nasale	na	The tip of the nasal bone
Nasion	n	The most anterior point of the frontonasal suture (if suture not clearly identified, then the deepest point on the nasal notch)
Opisthion	o	The midsagittal point on the posterior margin of the foramen magnum (at the saddle point)
Posterior nasal spine	pns	The apex of the posterior nasal spine
Prosthion	pr	The most anteroinferior point on the maxillary alveolar margin in the midsagittal plane
Sella	s	The center of the sella turcica

visible in each window, showed the current position in 3D and facilitated navigation through the CT data volume. Wireframe models (Netherway et al., 1995), developed to display the determined landmarks, aided visualization and comparisons.

Distances were calculated from the landmarks, and the asymmetry of selected bilateral distances was determined as a relative difference, expressed as a percentage:

$$\text{relative difference} = 100(\text{ipsi} - \text{contra})/[(\text{ipsi} + \text{contra})/2]$$

where "ipsi" and "contra" refer to ipsilateral and contralateral measurements. The ipsilateral side was defined as the side of the craniosynostosis or, for DP, the side of the occipital flattening. For some of the measurements, ipsilateral:contralateral ratios were calculated to facilitate comparisons with previous studies. The Student's *t* test was used to assess the significance of differences of the mean asymmetry from zero for each anthropometric variable.

Five DP and five UCS scans were randomly selected for repeat measurement for a double-determination study to assess the variance of landmark relocation (Abbott et al., 1990; Richtsmeier et al., 1995).

Generalized Procrustes and principal component analyses were applied directly to the landmark coordinate data (Dryden and Mardia, 1998; Rohlf, 1999, 2000). This procedure involved several steps. First, each patient's landmark configuration was standardized by translation to its centroid and scaled to unit centroid size. That is, it was scaled so that the sum of the squared distances from the centroid to each landmark was unity. Then, after simultaneous least squares (Procrustes) reg-

TABLE 4 Relative Difference in Percent Between Ipsilateral and Contralateral Measurements for Each Form of Plagiocephaly

Variable	DP (n = 21)			USC (n = 20)			ULS (n = 2)		
	Mean	SE	p	Mean	SE	p	Mean	SE	p
ans-hn	-6.0	0.48	.000*	-5.5	0.41	.000	-6.7	1.75	.164
ans-or	-5.8	2.94	.061	3.1	2.01	.142	7.4	14.50	.700
ans-po	-7.4	0.86	.000	-10.3	0.80	.000	-12.7	0.35	.018
cd-go	0.7	1.32	.609	-0.8	0.78	.341	-1.5	6.90	.864
cd-id	-4.0	0.36	.000	-4.5	0.47	.000	-10.0	3.85	.235
ct-cd	-5.9	1.11	.000	-4.9	1.38	.002	-11.1	4.25	.234
ct-go	1.0	0.79	.207	-1.0	0.54	.092	0.1	3.30	.981
ct-id	-1.5	0.53	.009	-4.7	0.57	.000	-9.3	6.00	.365
gn-cd	-2.3	0.32	.000	-3.5	0.42	.000	-7.3	3.70	.299
gn-ct	0.0	0.40	.962	-3.6	0.40	.000	-5.6	4.35	.423
gn-go	-3.2	0.65	.000	-2.6	0.55	.000	-7.0	3.05	.263
go-id	-4.5	0.69	.000	-2.5	0.45	.000	-7.7	3.10	.244
ilor-po	-3.1	0.64	.000	-16.8	0.86	.000	-17.0	2.55	.095
ilor-sl-or	-2.2	2.80	.436	23.0	3.21	.000	6.9	6.55	.486
iobf-or	4.3	2.89	.088	-3.0	2.72	.282	1.9	10.25	.886
mor-lor	-0.3	0.84	.745	-12.7	1.10	.000	-1.6	0.20	.079
n-ilor	-3.3	0.65	.000	-14.3	1.18	.000	-7.0	2.80	.242
n-or	-4.1	2.06	.059	-10.3	1.43	.000	-0.4	12.25	.982
n-po	-6.9	0.87	.000	-13.2	0.90	.000	-13.0	0.25	.012
n-sl-or	-3.8	0.75	.000	-11.3	1.18	.000	-5.5	1.05	.121
ofai-mor	2.2	1.30	.107	-1.1	2.23	.618	1.0	0.60	.344
ofai-or	5.8	0.77	.000	7.9	0.91	.000	10.9	2.75	.158
ofai-sl-or	11.0	0.98	.000	1.0	1.37	.457	12.2	3.00	.154
ofai-sl-or	6.2	0.87	.000	-5.9	1.01	.000	-0.5	0.15	.205
ofai-spc	5.1	1.42	.002	3.6	2.08	.103	0.5	0.55	.563
or-po	-5.5	0.96	.000	-15.4	0.77	.000	-19.2	4.50	.147
s-n-ba-ot†	-3.1	0.53	.000	-11.7	0.68	.000	-13.7	1.45	.067
sl-or-hn	7.2	1.00	.000	4.0	0.92	.000	2.9	3.20	.531
sl-or-po	-1.9	0.78	.024	-21.8	0.92	.000	-14.3	3.25	.143
sl-or-or	-1.1	1.19	.353	6.5	1.12	.000	0.9	7.50	.924
sl-or-or:sl-or-lor	-0.8	1.12	.480	19.1	1.55	.000	2.8	7.65	.780
spc-as	-4.8	0.78	.000	-12.5	0.99	.000	-21.0	3.75	.113
zt-po	-3.3	1.04	.005	-15.4	0.86	.000	-15.5	2.20	.090
zt-zf	-4.0	2.93	.189	13.9	4.40	.005	-9.5	9.40	.497

* $p = .000$ means $p < 5 \times 10^{-4}$.

† Angular difference in axial plane.

istration of the landmarks of each subject, the mean landmark configuration was determined as the average of the 3D positions of the registered homologous landmarks.

Principal component (PC) analysis was then carried out to express each patient's landmark configuration in terms of a sum of scaled standardized PC vectors. The scale factors are known as the standardized PC scores and are in units of standard deviations (SDs). Each component of a PC vector corresponds to a displacement at a landmark. The standardized PC vectors have magnitude equal to the square root of their contribution to the total variance and are ordered in descending contribution to the total variance.

Visualization of variations in shape represented by the PCs was achieved by warping (using thin plate spline functions) the mean shape along each PC (Rohlf and Bookstein, 2003). The visualization toolkit (Schroeder et al., 1996) was used to perform the GPA/PCA. The mean head shape was produced by taking the patient with the smallest full Procrustes distance from the mean and warping the skeletal surface so that the landmarks of that patient were registered exactly on the corresponding landmarks of the mean head shape. The full Procrustes distance of each patient from the mean head shape was calculated as the root-mean-square residual after registration

by rotation and scaling of the patient's landmarks onto the mean landmarks scaled to unit centroid size.

The statistics package "R" (<http://cran.us.r-project.org>; accessed June 2, 2005) was used for producing plots and for the descriptive statistics.

RESULTS

For each form of plagiocephaly (DP, UCS, and ULS) the mean, standard error (SE), and p value are given for 34 asymmetry variables (relative difference between ipsilateral and contralateral measurements) in Table 4. Figures 3 through 5 show selected asymmetry measures from Table 4 for each patient.

The SEs and p values for ULS were included as a guide because the small sample size precludes statistical conclusions regarding this group in isolation. Some comparisons were made among the groups (Table 5) by assuming equal variances and pooling variances. But observations regarding the ULS data are made with the proviso that an atypical value in our sample of two cases would have a substantial impact on extrapolation to ULS in general.

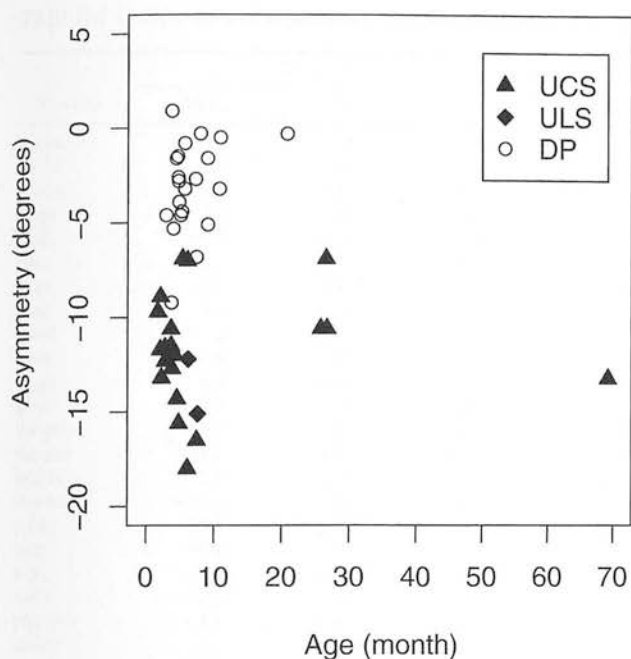


FIGURE 3 Deflection of the central axis of the endocranial base (the angle between the lines sella-nasion and opisthion-basion projected onto the axial plane) for each patient. The ipsilateral deflection was more severe, on average, for both UCS and ULS than for DP.

Accuracy of Measurements

For the 78 osseous landmarks used in this study, the double-determination analysis found the pooled (root-mean-square) and median landmark relocation errors to be 0.92 mm and 0.74 mm, with a range of 0.23 to 1.74 mm. Relocation errors were pooled over coordinate directions because sign tests indicated no significant differences, in spite of the higher resolution within CT slices than between them. The median landmark relocation error was approximately 1.5 pixels.

Asymmetry Variables

Many of the variables shown for UCS and DP in Table 4 are highly significant with probabilities of chance occurrence below 5×10^{-4} , indicating substantial measurable asymmetry throughout the craniofacial complex including the mandible. The cranial base angle (s-n-ba-o) showed mild twisting for DP and severe twisting for both UCS and ULS (Fig. 3). The orbital depths (ofai-sor) were significantly larger ipsilaterally for DP, contralaterally for UCS, but symmetric for ULS (Fig. 4). The orbits of patients with UCS were very distinctive with significant asymmetry of the orbital indices (sor-or: mor-lor). For each condition there was substantial asymmetry of the maxilla, evident in the maxilla depths (ans-hn), and of the mandible, evident in the mandibular lengths (gn-cd), although these were not statistically significant for the patients with ULS. Significant facial asymmetry was found in the facial depth distances (n-po) averaging -7% for DP, -13% for UCS, and -13% for ULS (Table 4). The negative sign indicates smaller ipsilateral

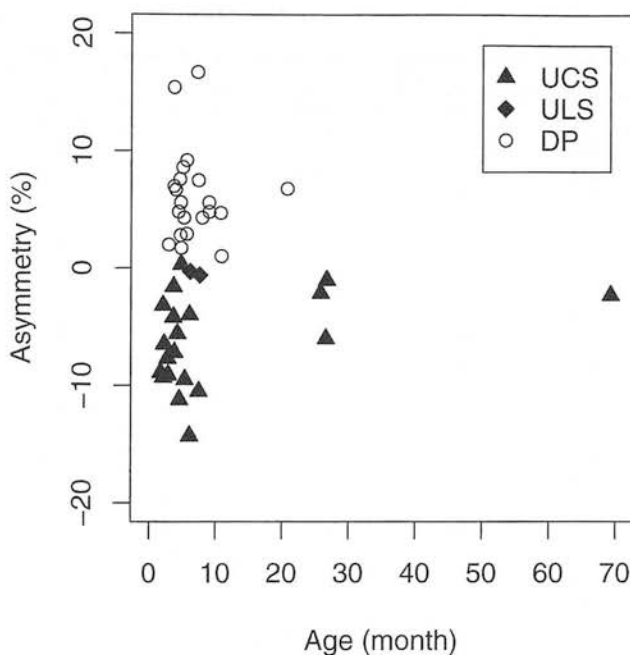


FIGURE 4 Relative asymmetry of the orbital depth measured from optic foramen anterior inferior (ofai) to superior orbitale (sor) for each patient. The orbital depths (ofai-sor) were significantly larger ipsilaterally for DP, contralaterally for UCS, but symmetric for ULS.

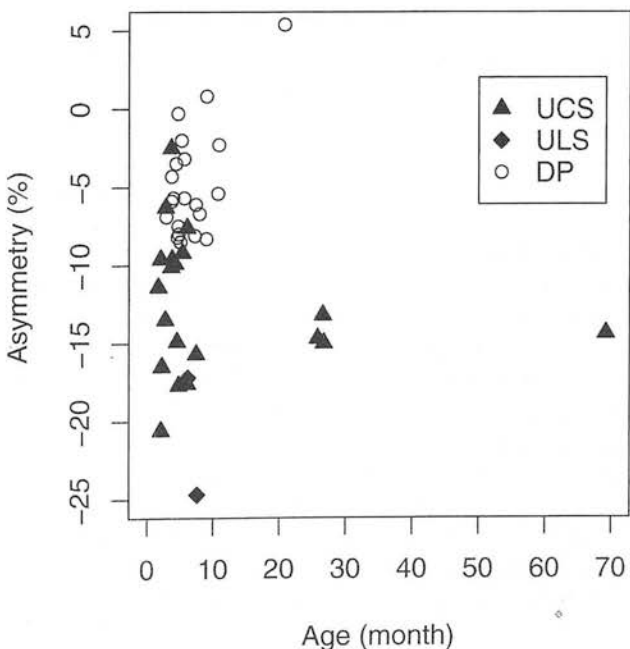


FIGURE 5 Relative asymmetry of the inferior parietal length measured from asterion (as) to sphenoion c (spc) for each patient. The mean asymmetry of the inferior parietal length (spc-as) differed significantly among the DP, UCS, and ULS groups.

TABLE 5 Comparison of asymmetry variables for UCS, ULS, and DP

Variable	DP (n = 21)			USC (n = 20)			ULS (n = 2)		
	Mean	SE	p	Mean	SE	p	Mean	SE	p
ans-hn	0.5	0.64	.419	-0.6	1.65	.719	-1.1	1.40	.432
ans-or	8.9	3.60	.018	13.2	10.29	.212	4.3	7.32	.561
ans-po	-2.9	1.18	.018	-5.3	2.86	.080	-2.3	2.58	.375
cd-go	-1.4	1.55	.358	-2.2	4.65	.643	-0.7	2.99	.807
cd-id	-0.5	0.59	.386	-6.0	1.48	.001	-5.5	1.78	.006
ct-cd	1.1	1.76	.539	-5.1	3.80	.194	-6.2	4.56	.190
ct-go	-2.0	0.97	.046	-0.9	2.72	.736	1.1	1.92	.586
ct-id	-3.2	0.78	.000*	-7.8	2.23	.002	-4.6	2.33	.064
gn-cd	-1.2	0.53	.031	-5.0	1.36	.001	-3.8	1.62	.031
gn-ct	-3.6	0.57	.000	-5.5	1.65	.003	-1.9	1.65	.256
gn-go	0.7	0.86	.455	-3.8	2.27	.114	-4.4	1.93	.034
go-id	2.0	0.84	.022	-3.2	2.40	.199	-5.2	1.64	.005
ilor-po	-13.7	1.06	.000	-13.9	2.18	.000	-0.2	2.84	.956
ilor-sl-or	25.2	4.25	.000	9.1	9.39	.345	-16.2	10.48	.139
iofb-or	-7.3	3.61	.050	-2.4	8.24	.770	4.9	9.13	.600
mor-lor	-12.4	1.38	.000	-1.3	2.77	.638	11.1	3.56	.005
n-ilor	-11.0	1.33	.000	-3.7	2.25	.114	7.3	3.88	.074
n-or	-6.2	2.53	.020	3.8	7.38	.614	10.0	5.44	.082
n-po	-6.3	1.25	.000	-6.0	2.88	.049	0.3	2.91	.924
n-sl-or	-7.5	1.38	.000	-1.6	2.49	.517	5.9	3.82	.138
ofai-mor	-3.3	2.55	.200	-1.2	4.31	.783	2.1	7.21	.771
ofai-or	2.1	1.18	.084	5.1	2.62	.067	3.0	3.00	.335
sfai-sl-or	-10.0	1.67	.000	1.2	3.30	.731	11.2	4.48	.022
sfai-sl-or	-12.1	1.32	.000	-6.6	2.87	.031	5.4	3.25	.112
sfai-spc	-1.5	2.50	.555	-4.6	4.71	.339	-3.1	6.72	.648
or-po	-9.9	1.23	.000	-13.7	3.33	.000	-3.8	2.69	.178
s-n-ba-ot	-8.6	0.86	.000	-10.6	1.78	.000	-2.0	2.22	.387
sl-or-hn	-3.2	1.36	.024	-4.3	3.39	.215	-1.1	3.07	.715
sl-or-po	-19.9	1.21	.000	-12.3	2.70	.000	7.5	3.08	.024
sl-or-or	7.6	1.63	.000	2.0	4.29	.641	-5.6	4.01	.178
sor-or-mor-lor	19.9	1.90	.000	3.6	4.10	.395	-23.4	14.39	.119
spc-as	-7.7	1.25	.000	-16.2	2.71	.000	-8.5	3.31	.019
zl-po	-12.1	1.36	.000	-12.2	3.48	.002	-0.1	2.82	.973
zt-zf	17.9	5.24	.001	-5.5	9.92	.584	-23.4	14.39	.119

* .000 means $p < 5 \times 10^{-4}$.

† Angular difference in axial plane.

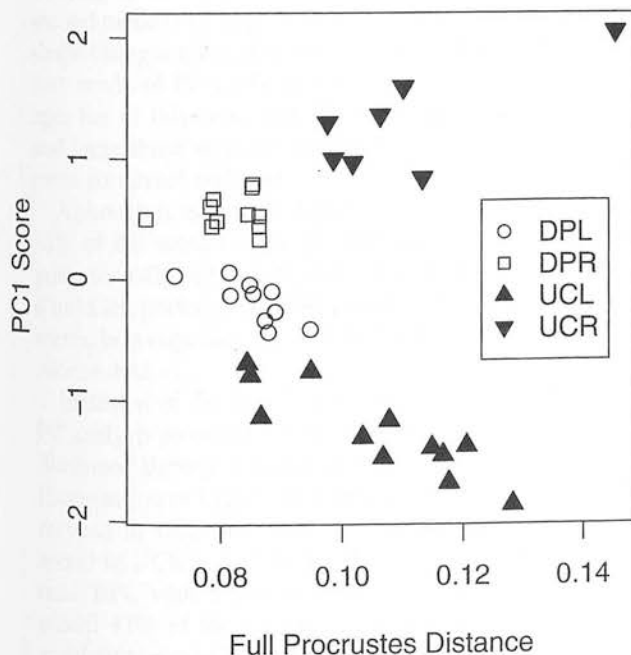


FIGURE 6 Two variables from the GPA/PCA analysis were sufficient to distinguish the plagiocephaly phenotype and the affected side: left DP (DPL), right DP (DPR), left UCS (UCL), and right UCS (UCR).

size. For ULS, the asymmetry of the inferior parietal length (spc-as) was significantly more extreme than for both DP and UCS groups (Table 4 and Figure 5).

PC Analysis

Generalized Procrustes and principal component analyses were first applied to only the DP and UCS data, excluding the data of the two patients with ULS. Landmarks associated with the mandible were excluded from the GPA/PCA because of the varying position of the mandible as a result of intubation. The scatter plot (Fig. 6) of the first PC (PC1) score against the full Procrustes distance (the root-mean-square of the residuals after least squares registration with the mean landmark configuration) illustrates the separation of the data by diagnosis and the ipsilateral side. The PC1 score in the scatter plot is the contribution of the first PC or mode to each individual patient's head shape in units of the SD for that mode. The first 10 PCs explain 75% of the variation.

Figures 7 and 8 illustrate the effect of the first two modes on the mean head shape by applying a variation of ± 2 SDs along these principal directions of shape change. The first two PCs appeared to represent UCS and posterior DP. The first

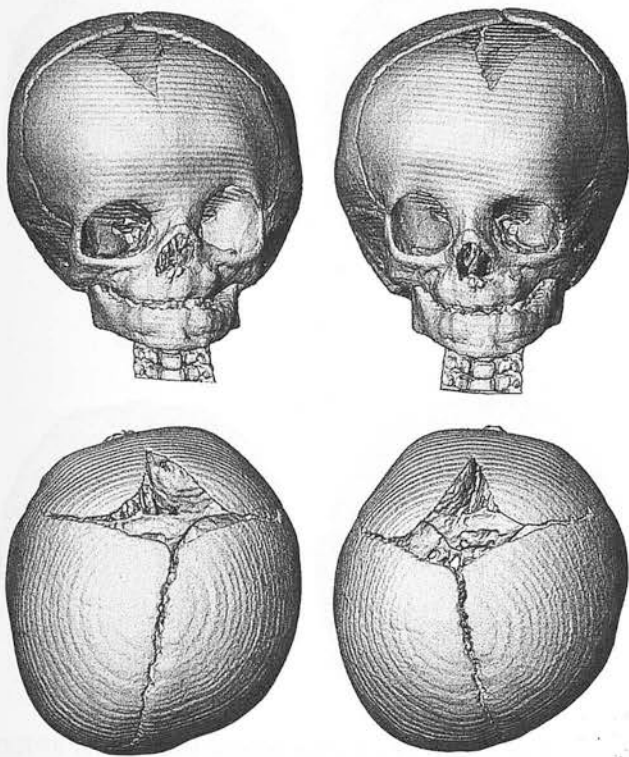


FIGURE 7 The first principal mode of plagiocephalic shape change shown by warping the mean head shape -2 SDs (left) and $+2$ SDs (right) along the first principal direction. This mode was dominated by shape changes related to dystopia, twisting of the sagittal suture, and the frontal bone.

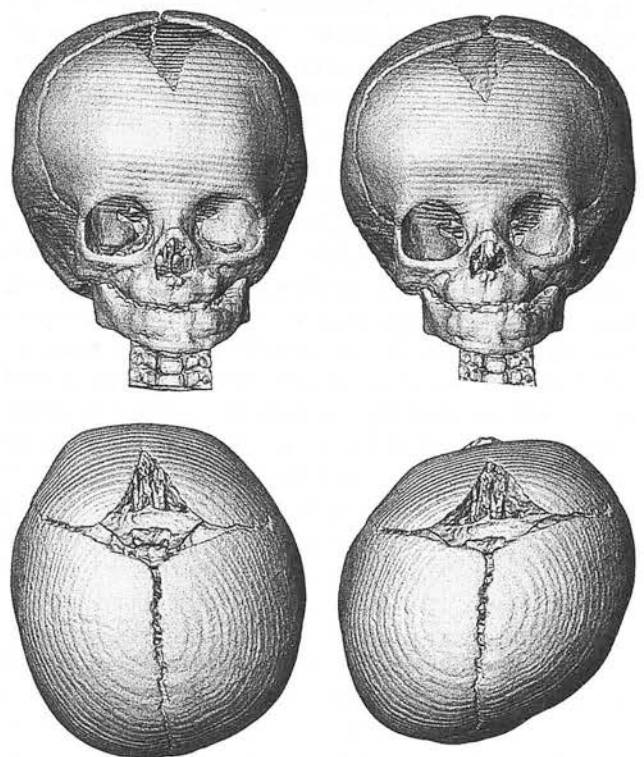


FIGURE 8 The second principal mode of plagiocephalic shape change shown by warping the mean head shape -2 SDs (left) and $+2$ SDs (right) along the second principal direction. This mode was dominated by shape changes related to twisting of the cranium without twisting of the cranial base and sagittal suture.

mode (Fig. 7) gave severity in terms of degree of dystopia and twisting of the cranial base and sagittal suture, whereas the second mode (Fig. 8) gave severity in terms of mostly calvarial shape changes without twisting of the sagittal suture. Often the first mode of PC analysis is strongly correlated with size or age, but in this case, with the relatively small spread in age and large shape variation because of the conditions, PC3 is the most correlated with age.

Although it is clear in Figure 8 that the head shape at $+2$ SDs of the second mode has the appearance of severe right posterior DP, the head shape at -2 SDs has the appearance of a mild left posterior DP. This indicated that there was an asymmetric bias regarding affected side and severity in the available patient data.

Inclusion of the data for the two cases of right ULS in the PC analysis produced similar distinction of the conditions best illustrated through a scatter plot of PC3 against PC1 (Fig. 9). Examination of Figure 9 shows that the extremes in PC1 correspond to UCL and UCR, whereas extremes in PC3 correspond to UCR and ULR. Extremes of PC2 (not shown) contrast DPL with the other conditions. The first three modes model 47% of the sample variation, whereas 11 modes are required to model 75% of the variation. The first two principal modes appeared very similar to those shown in Figures 7 and 8, whereas the third mode showed flattening of the right occiput for positive scores corresponding to right lambdoid syn-

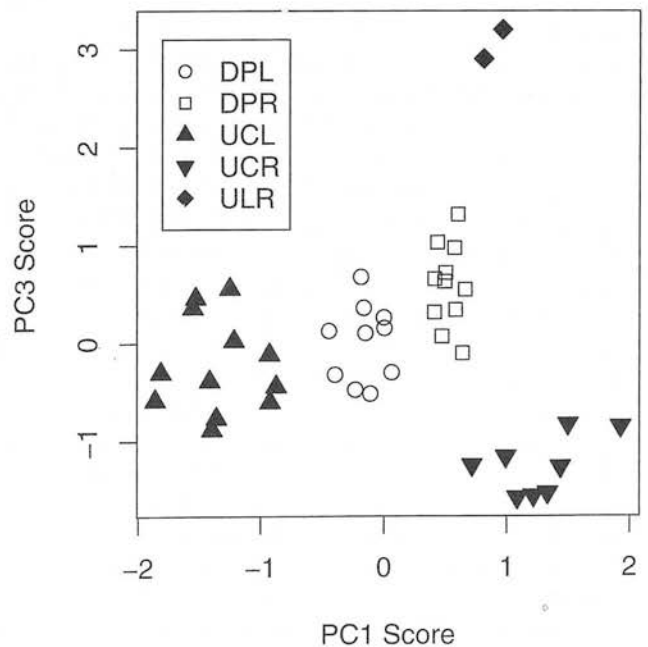


FIGURE 9 Separation of the plagiocephaly phenotypes (DP, UC, and UL) and affected side (L or R) was evident in this two-dimensional scatter plot of the contribution of the first and third principal modes of shape variation to the head shape of each patient.

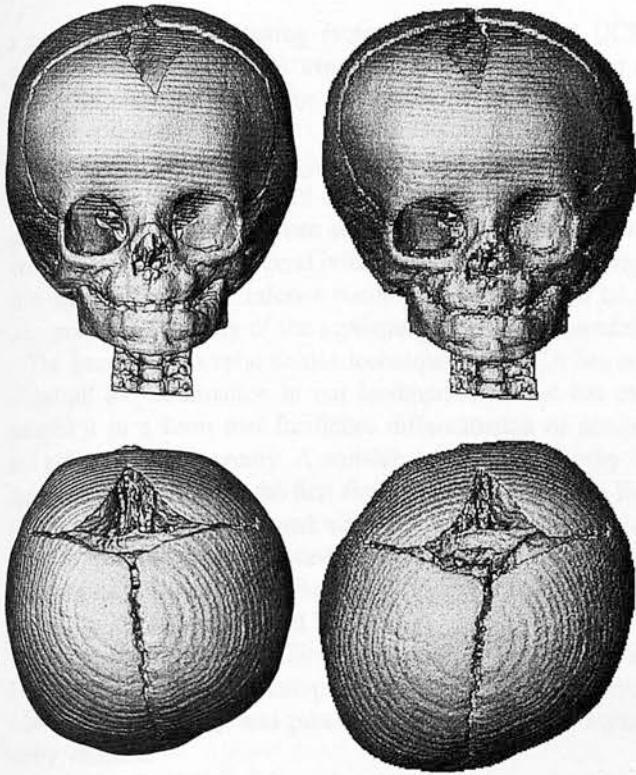


FIGURE 10 The third principal mode of plagiocephalic shape change shown by warping the mean head shape -2 SDs (left) and $+2$ SDs (right) along the third principal direction. This mode displayed flattening of the right occiput for positive scores corresponding to right lambdoid synostosis.

ostosis (Fig. 10). The two cases of right lambdoid synostosis have PC3 scores at approximately $+3$ SDs.

DISCUSSION

The distinct head shapes formed by nonsyndromic cranio-synostosis result from restricted growth at the fused suture and compensatory growth at the patent sutures, whereas positional molding forces explain the shape of DP (Huang et al., 1996; Cohen, 2000).

This investigation has quantified statistically significant asymmetry in a variety of craniofacial parameters of infants who had untreated plagiocephaly. The rarity of lambdoid synostosis meant that only two cases were available for comparison. Although this was insufficient for statistical analysis, the two cases were included to find where their parameters fell within the range of variation for DP and UCS. The two cases of lambdoid synostosis differed from other reported descriptions of ULS by the position of the ipsilateral ear (Menard and David, 1998). That is, there was anterior displacement of the ipsilateral ear position rather than the generally observed posterior displacement (Huang et al., 1996). This meant that our measurements yielded negative asymmetry for ans-po distances, indicating a smaller ipsilateral distance.

Previous measurements of the range of dysmorphology in plagiocephaly have been of the mandibular asymmetry (Kane

et al., 1996), the endocranial base angle (Lo et al., 1996b), and the orbits in UCS (Lo et al., 1996a). This study has extended the measurement of the asymmetry and performed some geometric morphometrics by GPA/PCA.

Mandibular measurements (e.g., gn-cd) were found to have significant asymmetry both for patients with UCS and for patients with DP, and similar asymmetry was evident in the ULS group. These results for the UCS group were consistent with those found by Kane et al. (1996), that is, body shortening on the affected side without significant involvement of the ramus. However, shortened ramal height (cd-go) was found in their DP group but not in DP group in this study. The ipsilateral body shortening suggested that the mandible is twisted in the ipsilateral direction. On average, significant diminution was found for DP and UCS for the ipsilateral distance between condylion and the coronoid process (ct-cd). This could potentially be linked to distortion of the temporal aspect of the intimately linked zygoma. Supporting this was the finding of ipsilateral diminution of the zygomal length (zt-po).

When comparing the present results with those of Kane et al. (1996), a few methodological differences should be noted. For example, the distance from pogonion to condylion (the cephalometric landmark positioned at the most superior point of the condylar head) was used in their study to assess asymmetry of the length of the mandible, whereas gnathion and condylion laterale were used in the present study. Nevertheless, the asymmetry measures in terms of relative bilateral differences would be expected to be similar. For example, the asymmetry of the mandibular length (gn-cd) for DP and UCS were -2.3 ($p < .001$) and -3.5 ($p < .001$) in this study and 0.6 ($p = .32$) and -3.5 ($p < .001$) in their study after taking into account the sign differences between the two methodologies.

As in the study by Lo et al. (1996b), severe deviation of the midlines of the anterior and posterior cranial fossae in the axial plane for both UCS and ULS were found. For DP, a small but significant ipsilateral deflection of the central axis of the endocranial base was found (3° on average). This contrasts with Lo et al. (1996b), who found, for their DP study sample, "the direction of this deviation inconsistent with respect to occipital flattening." There was no angular overlap between their DP group and synostosis groups (a 7° boundary was indicated), whereas for the current study groups there was overlap: the maximum for DP was 9.2° and the minimum for UCS was 6.9° . This simply indicated that there was a more severe case of DP in the present sample. The finding of significant measurable ipsilateral twisting of the central axis of the endocranial base for patients with DP was novel and added to the measurable impact of the transmitted asymmetric forces.

The orbital dysmorphology in UCS was one of the most characteristic features of the condition, displaying on average a 19% larger ipsilateral orbital index (sor-or: mor-lor), whereas both DP and ULS were found to have symmetric indices. Orbital roof measurements were found useful to describe the asymmetry of the anterior cranial fossa: the distance between the optic foramen and superior orbitale was found to provide

a contributing differentiating factor between DP and UCS, with a notable increase on average observed for DP and a similar decrease for UCS. Our two values for ULS were symmetric for this variable.

Comparison of specific asymmetry variables has provided an appreciation of the impact of each condition on a variety of features, but there has been some loss of information. For example, neither the ipsilateral inferior tilt of the foramen magnum nor the ipsilateral inferior positioning of the ear in ULS was represented by any of the asymmetry variables presented.

The geometric morphometrics technique GPA/PCA has retained all the information in our landmark data and has expressed it in a form that facilitates differentiation of groups and assignment of severity. A suitable measure of severity is the root-mean-square of the first few PC scores, excluding the PC corresponding to age and size. For example, this is the distance from each point plotted in Figure 9, corresponding to each patient in the study, to the origin (except that for the first 3 PCs the 3D distance would be used).

The discrimination capability of PC analysis can be seen in Figure 9 where there is complete separation of the groups, whereas some overlap was present for each individual asymmetry variable.

For both GPA/PCA analyses (with and without inclusion of the ULS data), the patient with head shape closest to the mean was the one shown in Figure 2. Although the skeletal surface data were warped to match the mean landmark configuration, some residual asymmetry was evident. The unequal numbers of left and right affected individuals and an asymmetric distribution of severity would have contributed to this residual asymmetry.

As previously mentioned, the three types of plagiocephaly described here can usually be differentiated through physical examination and diagnostic imaging. The main contribution of this project was to show that the relatively new GPA/PCA procedures would be able to quantify severity in terms of asymmetry and shape differences and that it could differentiate the phenotypes of plagiocephaly.

In the future, analysis of asymmetry and GPA/PCA may help facilitate differentiation of subtle phenotypic differences among closely related genotypes. In addition, there may be application to following these measures of craniofacial shape for quantifying alteration as a result of surgical intervention.

More immediate work will include incorporation of data from more patients in the groups presented here, other conditions, and skeletally normal children.

CONCLUSIONS

In addition to the cranial asymmetry evident in patients with plagiocephaly, significant asymmetries were found and quantified in the cranial base and facial structures through the use of angular and distance measures between landmarks located in 3D. Although there was overlap of measurements for a single 3D cephalometric variable for at least two of the three forms of plagiocephaly, the GPA/PCA approach was able to

distinguish among DP, UCS, and ULS. The impact of unilateral craniosynostosis and deformational forces on craniofacial shape were visualized by warping a mean head shape along the first few principal directions of shape variability. For each patient, the first few PC scores, excluding the PC corresponding to age and size, could be used as indicators of severity.

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ORIGINAL ARTICLES

A Three-Dimensional Computed Tomographic Analysis of the Cervical Spine in Unoperated Infants With Cleft Lip and Palate

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Objective: To investigate anatomical variations and abnormalities of cervical spine morphology in unoperated infants with cleft lip and palate.

Design: Retrospective cross-sectional investigation of infants born with non-syndromic cleft lip and palate using computed tomography scans acquired for investigation of a spectrum of clinical conditions.

Setting: Computed tomography scan data were obtained from 29 unoperated cleft lip and palate infants and 12 noncleft infants of Malay origin, ages 0 to 12 months.

Methods: Observational study of cervical spine computed tomography scans. Heights of cervical vertebral bodies (C2-C7) and intervertebral spaces were measured from landmarks identified from computed tomography reformats and three-dimensional computed tomography reconstructions. Linear modeling of heights and spaces, with age as a covariate, was undertaken to identify differences between the samples.

Results: Anomalous features observed in the cleft lip and palate sample included short posterior arch of C1 (2/29), abnormal development of the anterior arch of C1 (2/29), and fusions of the posterior arch of C2 and C3 (2/29). No anomalies of the cervical spine were observed in the noncleft sample. Although the heights of three cervical vertebral bodies were significantly smaller and two intervertebral spaces were significantly larger in infants with cleft lip and palate compared with noncleft infants ($p < .05$), overall length of the cervical spine did not differ significantly between the samples.

Conclusion: There was evidence for subtle upper spinal anomalies in the infant cleft lip and palate population. Our finding of reduced size of some cervical vertebral bodies may reflect delayed upper spinal development in infants with cleft lip and palate.

KEY WORDS: *cervical spine anomalies, cleft lip and palate, computed tomography*

Cleft lip and palate (CLP) is the most common congenital craniofacial deformity in humans, affecting one in every 700

to 1000 newborns worldwide (Murray, 1995, 2002). Previous studies have identified that infants with oral clefts often have other subtle, associated congenital defects (Shprintzen et al., 1985; Leth-Jensen et al., 1988; Hagberg et al., 1997; Stoll et

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TABLE 1 Age and sex distribution of the CLP and NC groups

Group	Sex	n	Mean Age (d)	SD	Range min–max
CLP	Girls	12	29	115	76
	Boys	17			
NC	Girls	3	12	145	86
	Boys	9			

al., 2000), including cervical spine anomalies (Sandham, 1986; Horswell, 1991; Ugar and Semb, 2001). Furthermore, Osborne et al. (1971) reported associations between malformations of the cervical spine and velopharyngeal incompetency in CLP individuals.

Most previous studies of cleft lip and palate have applied two-dimensional lateral cephalometric methods. However, this approach has significant limitations, such as superimposition of structures, difficulty in identifying landmarks, and poor visualization of three-dimensional (3D) structures (Moyers and Bookstein, 1979; Helmi and Pruzansky, 1980; Cohen, 1984; Fisher et al., 1999). Furthermore, subjects in these studies have been older children and adults, and the investigations have been limited to specific ethnic groups. Researchers have recognized the potential advantages of using 3D computed tomography (CT) to clarify whether CLP is associated with other craniofacial malformations or is expressed only as a localized anomaly (Maue-Dickson and Dickson, 1980; Mølsted et al., 1995). However, we are not aware of any previous CT studies of the cervical spine in CLP infants during their first year of life before any surgical intervention has occurred.

The aim of this study was to use CT imaging to compare the heights of individual cervical vertebral bodies, intervertebral spaces, and overall length of the cervical spine between four groups of infants with clefts: unilateral cleft lip and palate (UCLP); bilateral cleft lip and palate (BCLP); isolated cleft palate (ICP); and cleft lip and primary palate/alveolus (CL); and an unaffected, noncleft (NC) group. Furthermore, we aimed to describe anatomical variations and abnormalities of cervical spine morphology in the CLP sample.

METHODS

Patients

The study sample consisted of 29 unoperated nonsyndromic children of Malay origin, ages 0 to 12 months, with CLP who had 3D CT examinations during the period January to December 2002. CT scans for 12 NC Malay patients of the same age range were collected over the same period. These patients had presumed normal craniofacial morphology, but had indications for CT scanning for problems other than those recognized to cause abnormalities in craniofacial growth and morphology. Ethical approval was obtained from the Research and Ethics Committee, School of Medical Sciences, Universiti Sains Malaysia, for access to the data used in this study.

The distribution of clefts was as follows: CL, $n = 7$; UCLP,

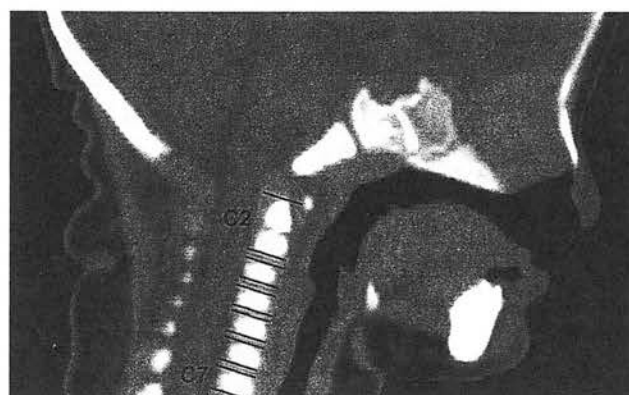


FIGURE 1 Heights of vertebral bodies and thicknesses of intervertebral spaces were measured from C2 to C7.

$n = 10$; BCLP, $n = 4$; ICP, $n = 8$; NC, $n = 12$. Cephalometric analyses have shown that individuals with CL (cleft of the primary palate) differ in craniofacial morphology from other cleft types (Dahl, 1970), so a separate CL category was included in this study. Age and sex distribution of the CLP and NC groups are shown in Table 1. A few older children were included in the CLP group, because their primary operation had been postponed due to other health problems, such as upper respiratory tract infection and aspiration pneumonia.

CT Data Acquisition

Helical scans were obtained with a GE Lightspeed Plus CT Scanner System (GE Healthcare Technologies, Waukesha, WI) at the Department of Radiology, Hospital Universiti Sains Malaysia. The tube voltage and current that were used were 120 kV and 120 mA, respectively, but minor adjustments to these parameters were made according to the patient's size. Axial slices of 1.25 mm thickness with a spacing of 1.25 mm were written to CD for transfer to a workstation for measurement.

The PERSONA software package developed at the Australian Craniofacial Unit, Adelaide, Australia (Netherway et al., 1999; Abbott et al., 2000), was used to create 3D CT reconstructions and to determine the 3D coordinates of osseous landmarks on a Silicon Graphics Octane workstation (SGI, Mountain View, CA).

Cervical Spine Anomalies

The CT reformats and 3D CT reconstructions were examined to identify cervical spine anomalies such as fusion of the posterior elements of the cervical vertebrae and abnormal development of C1.

Measurement

Landmarks were positioned at the anterior superior medial surface and the anterior inferior medial surface of each of the vertebral bodies (C2–C7). Vertebral body and intervertebral space heights were determined from these landmarks (Fig. 1).

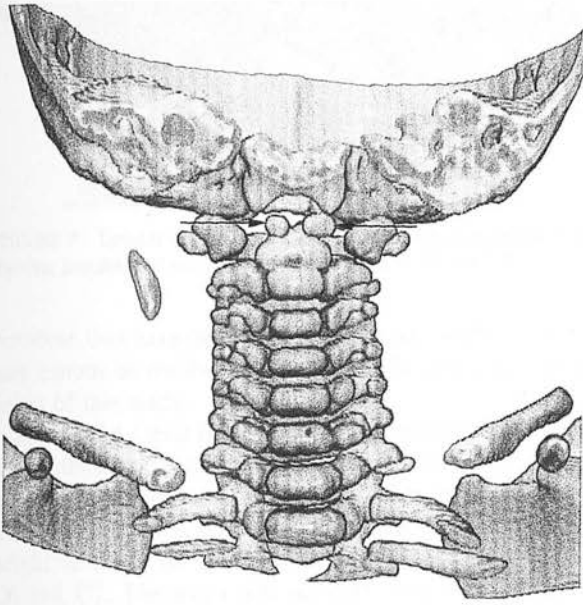


FIGURE 2 Anterior view of a 3D CT reconstruction of an infant with UCLP showing separation of the anterior tubercle of C1.

The total length of the cervical spine was measured from the anterior inferior medial surface of C7 to the anterior superior medial surface of C2.

Statistical Analysis

A linear model (PROC GLM, SAS/STAT User's Guide, Version 8, SAS Institute Inc., Cary, NC) incorporating the fixed effects of sex (male, female) and cleft group (NC, UCLP, BCLP, ICP, CL), and using age (14 to 340 days) as a covariate, was fitted to all cervical measurements: measurement \sim age + sex + group.

Higher-order interactions were not analyzed for this small data set. Linear contrasts were arranged to compare the control group (NC) with all other groups, and to compare the ICP group (a distinct cleft type) with other cleft groups.

Error Analysis

Repeat determinations were performed for all infants after a period of 1 month to assess the reproducibility of landmark determination and variables derived from these landmarks. Student's paired *t* tests were used to detect systematic errors (i.e., to ascertain whether the mean difference between repeated measures deviated significantly from zero) and the Dahlberg (1940) method of double determination was used to quantify the magnitude of random errors.

RESULTS

Cervical spine anomalies were found in three of the infants with CLP (10%). None of the NC group showed cervical spine anomalies.

Of the CLP infants with visible ossification of the anterior

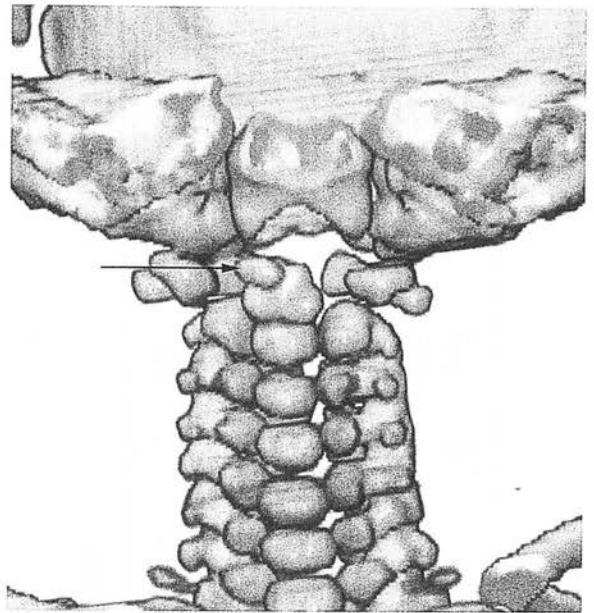


FIGURE 3 Anterior view of a 3D CT reconstruction of an infant with ICP showing asymmetry of the anterior tubercle of C1 (located to the right of the midline).

arch of C1, anomalies were observed for two CLP infants. Ossification of the anterior arch of C1 was visible in 12 of 29 (41%) of infants with CLP and in 5 of 12 (42%) of infants in the NC sample. Ossification was detected if the voxel density was greater than 150 Hounsfield. The anomalies of the anterior arch of C1 for the CLP group comprised bifurcation of the anterior tubercle of C1 (Fig. 2) and an asymmetric position of the anterior tubercle with displacement to the right (Fig. 3).

The posterior arch of C1 was underdeveloped for two CLP infants (e.g., Fig. 4). The 3D CT reconstruction in Figure 4 also shows apparent fusion of the posterior arches of C2 and C3. If adjacent structures are separated by less than the CT slice thickness (1.25 mm in this study), they can appear fused. Although this image, and the one shown in Figure 5, appear to show the onset of fusions of the arches of the upper spinal

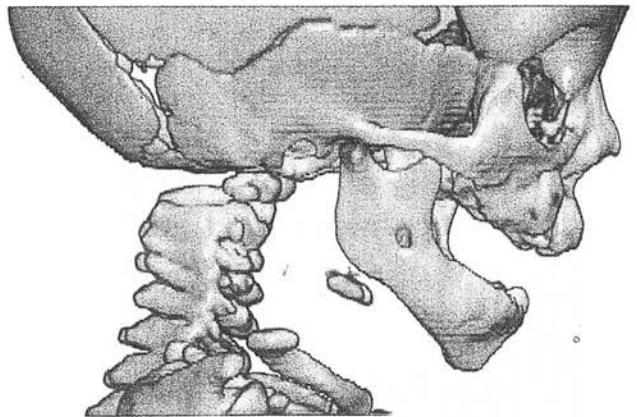


FIGURE 4 Lateral view of a 3D CT reconstruction of an infant with UCLP showing underdeveloped posterior arch of C1.

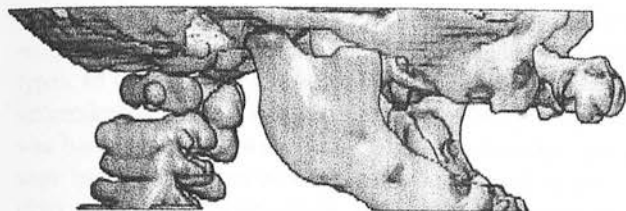


FIGURE 5 Lateral view of a 3D CT reconstruction of an infant with CLP showing possible fusion of the posterior arches of C2 and C3.

vertebrae that have been observed in older children with CLP, they cannot be resolved definitively in infants using the resolution of this study.

The paired *t* tests between repeat determinations of the vertebral distances indicated no systematic errors. The random measurement error as given by the Dahlberg statistic derived from the repeated determinations, ranged from 0.2 mm for the height of C2 to 0.5 mm for the intervertebral space between C6 and C7. The mean measurement error was 0.4 mm, approximately the size of one pixel within a CT slice. These findings indicated that measurement errors were small and unlikely to bias the results.

The adjusted means and standard errors derived from the linear modeling analysis of vertebral distance data in the four cleft groups and the NC group are shown in Table 2. The lower vertebrae were not always within the range of the CT scan, hence the reduced numbers indicated in the table for their measurements. Differences in measurement method and sample size variation account for the discrepancies between the total length C2-C7 and the combined dimensions of vertebral bodies and intervertebral spaces.

The adjusted means in Table 2 take sex into account, although no significant differences were found between measurements taken from boys versus girls. The vertebral body heights of C3, C4, and C7 in CLP infants were found to be significantly smaller than in the NC group ($p < .05$). In contrast, the intervertebral spaces between C4/C5 and C5/C6 in CLP infants were significantly greater compared with the NC group ($p < .05$). The intervertebral spaces of C5/C6 in the ICP group were significant smaller, as compared with the other cleft groups ($p < .05$). Even though the CLP groups displayed smaller individual vertebral heights, the average overall lengths of their cervical spines were found not to differ significantly from the NC group (Table 2).

DISCUSSION

Many authors have noted relationships between facial malformations and spinal anomalies (Sherk et al., 1982; Anderson et al., 1997) that are thought to result from the close spatial relationship between sclerotomic derivatives of the cervical somites and the branchial arches (Sherk et al., 1982). Our findings indicate that upper cervical spine anomalies in Malaysian children with CLP (10%) are similar to the findings of other studies of children with CLP: American (22%; Horswell, 1991), Scottish (13%; Sandham, 1986), and Norwegian (18%;

TABLE 2 Adjusted means and standard errors (mm) for the heights of the vertebral bodies and intervertebral spaces (IVS)

Measurements	NC			UCLP			BCLP			ICP			CL		
	<i>n</i>	\bar{x}	SE	<i>n</i>	\bar{x}	SE	<i>n</i>	\bar{x}	SE	<i>n</i>	\bar{x}	SE	<i>n</i>	\bar{x}	SE
Height C2	12	13.0	0.40	10	13.0	0.43	4	13.1	0.68	8	13.5	0.48	7	13.1	0.50
IVS C2/C3	12	3.1	0.23	10	3.3	0.25	4	3.1	0.39	8	2.9	0.27	7	3.2	0.29
Height C3*	12	4.4	0.16	10	3.7	0.17	4	3.1	0.27	8	4.0	0.19	7	4.2	0.20
IVS C3/C4	12	2.5	0.18	10	3.0	0.19	4	2.9	0.30	8	2.7	0.21	7	2.7	0.22
Height C4*	11	4.5	0.19	10	3.8	0.19	4	3.8	0.30	8	4.0	0.21	7	4.3	0.22
IVS C4/C5*	11	2.3	0.16	10	3.3	0.16	4	3.1	0.25	8	2.6	0.18	7	2.6	0.19
Height C5	11	4.6	0.16	10	3.9	0.16	4	4.0	0.25	8	4.5	0.18	7	4.5	0.19
IVS C5/C6*	11	2.5	0.18	10	3.2	0.19	4	3.2	0.30	8	2.5†	0.21	6	2.9	0.24
Height C6	11	4.7	0.20	10	4.3	0.21	2	4.2	0.48	8	4.6	0.23	6	4.6	0.26
IVS C6/C7	9	2.9	0.18	9	3.2	0.17	2	3.1	0.35	8	2.8	0.17	6	3.1	0.19
Height C7*	5	5.3	0.26	4	4.6	0.26	1	3.8	0.54	6	4.5	0.20	4	4.8	0.25
Length C2-C7	5	41.4	1.50	4	45.0	1.50	1	38.6	3.05	6	45.0	1.14	4	44.5	1.44

* Significant difference at $p < .05$ between all cleft groups and the noncleft group; highlighted in bold.

† Significant difference at $p < .05$ between ICP and the other cleft groups; highlighted in bold.

Ugar and Semb, 2001). However, it must be stressed that the referenced study groups include different proportions of cleft types, so comparisons of frequencies of occurrence should be undertaken with some caution. Furthermore, the present study was based on 3D CT scans of infants, whereas earlier studies were based on two-dimensional cephalometric radiographs of older children. Previous studies have reported frequencies of fusion in NC groups, ranging from 0.5% to 5% (Brown et al., 1964; Gray et al., 1964; Farman and Escobar, 1982; Ugar and Semb, 2001). Although we did not find any fusion anomalies in our NC group, this may be due to the small sample size or perhaps to differences between ethnic groups.

It has been reported that congenital fusion of the cervical spine is due to the failure of normal segmentation of cervical somites *in utero* (Hensinger, 1990). In addition, another study reported that deficiencies of the disc-like material between the cartilaginous hemicentra might favor bony fusion (Muller et al., 1986). Congenital fusion of the cervical spine has been associated with clinical sequelae in Klippel-Feil syndrome, where cleft palate is also a frequently associated finding, together with short neck and low posterior hairline (Helmi and Pruzansky, 1980).

The anterior arch of C1 and the upper cervical vertebrae appear to be important in achieving adequate velopharyngeal closure and speech, because the musculofascial layer that covers them and forms the posterior pharyngeal wall is only 2 to 5 mm thick (Berkowitz, 1996). Indeed, epidemiological studies have shown that patients with craniofacial birth defects, many of whom suffer velopharyngeal incompetence, have a higher prevalence of upper cervical spine anomalies than the general population have (Osborne et al., 1971).

The anomalies of C1 found in this current study suggest a predictive role for C1 in the management of children with CLP, particularly in relation to speech. The emerging importance of the development of C1 as an early indicator of craniofacial growth in NC subjects also has been highlighted by previous studies (Huggare, 1989; Solow and Siersbaek-Nielsen, 1992).

It has been suggested previously that a lower age limit of 6 years should be set for the identification of cervical anomalies, because malformations of the upper cervical vertebrae cannot be assessed using conventional radiography until complete development has occurred (Sandham, 1986). Indeed, Sandham (1986) and Ugar and Semb (2001) excluded CLP patients younger than 6 years of age in their studies for this reason. However, the use of 3D CT technology enabled us to observe the presence of ossification and anomalies of the cervical spine in CLP at an earlier stage of childhood.

We found shortening of individual cervical vertebral bodies in CLP infants, compared with an NC group. Intervertebral spaces tended to be larger in CLP infants than in NC infants, except for the ICP group, which was smaller than other affected groups. These changes may relate to an altered ossification pattern or skeletal development of the cervical spine in the cleft individuals. Indeed, the finding of short vertebral bodies in the cervical spines of infants with clefts is consistent with a general delay in growth in infancy in this condition that

has been reported by others (Bowers et al., 1987; Neiman and Savage, 1997; Spyropoulos and Burdi, 2001). Although our finding for overall cervical spine length differs from that of Šmahel and Skvarilova (1993), who found, using lateral head radiographs, shortening of the overall length of the cervical spine in UCLP and BCLP groups, the subjects in their study were adults who had been treated surgically.

CONCLUSIONS

This study of unoperated CLP infants younger than 1 year of age provided evidence for a range of upper spinal anomalies, including smaller vertebral bodies, larger vertebral spaces, underdeveloped posterior arch of C1, and asymmetric position and bifurcation of the anterior tubercle of C1. This is further evidence that there may be subtle cervical spine anomalies associated with CLP in infants.

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ORIGINAL ARTICLES

The Hyoid Bone in Malay Infants With Cleft Lip and Palate

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Objective: To compare morphological and positional variations of the hyoid bone in unoperated infants with cleft lip and palate (CL/P) with those in noncleft infants.

Design: Retrospective, cross sectional.

Patients and Methods: Three-dimensional computed tomography scans were obtained from 29 unoperated CL/P infants of Malay origin aged between 0 and 12 months and from 12 noncleft infants in the same age range. Observations were made and measurements were obtained with a software package developed at the Australian Craniofacial Unit. The sizes of the hyoid bones and the position of the hyoid body and epiglottis in relation to the cervical spine were measured. Anatomical anomalies of the hyoid and prevalence of aspiration pneumonia were also documented.

Results: The hyoid bones and epiglottis were found to be located more inferiorly in CL/P infants compared with the noncleft infants. Also, 17% (5/29) of the CL/P infants had nonossified hyoid bodies.

Conclusion: The results suggest that there are differences in the location and genesis of the hyoid bone in infants with CL/P that warrant further investigation.

KEY WORDS: aspiration pneumonia, cleft lip and palate, hyoid bone anomalies, three-dimensional computed tomography

The hyoid bone has no bony articulation. The floor of the mouth and the tongue lie above it, the larynx below, and the

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epiglottis and pharynx posteriorly. Important muscles are attached to the hyoid bone, with a range of functions including maintaining the airway, swallowing, and preventing regurgitation. Problems with these vital functions may be associated with variations in position of the hyoid bone in relation to the craniofacial structures and head posture (Tallgren and Solow, 1987; Athanasiou et al., 1991).

The morphology and position of hyoid bone in cleft lip and palate (CL/P) are not well described, presumably because of difficulties in visualization by conventional radiographic techniques. We are aware of only one previous study (Kaduk et al., 2003) that has compared the position of the hyoid bone in CL/P patients with the position in noncleft (NC) individuals.

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By lateral cephalometry, the authors found that the hyoid bone in their CL/P patients was located more caudally (inferiorly) and anteriorly than in NC subjects and hypothesized that this may be associated with the malformation. Their CL/P patients differ from those of our study in that they were older children of European descent with repaired clefts.

Alteration in the morphology and position of the hyoid bone presents significant potential problems in terms of breathing, swallowing, and head posturing because of alterations in the attachment and pull of the muscles responsible for these functions. However, up until now, it has been unclear whether anatomical variations of the hyoid in CL/P infants have been associated with *in utero* disturbances or postnatal surgical corrections. For these reasons, and also because of the major advances in computed tomography (CT) imaging and computer technology, we have carried out the first detailed three-dimensional (3D) CT study of this important structure in unoperated CL/P infants compared with a group of NC infants in the same age range.

Specifically the objectives were to (1) quantify anatomical variations of the hyoid bone; (2) compare the anatomical length and height of the hyoid bone between unoperated CL/P and NC infants; (3) compare the position of the hyoid bone and epiglottis in relation to the cervical vertebrae; and (4) relate the findings to clinical problems, such as aspiration pneumonia.

METHODS

Patients

The patient sample consisted of 29 unoperated CL/P infants of Malay origin aged between 0 and 12 months requiring 3D CT examinations during the period January to December 2002. A GE Lightspeed Plus scanner (GE Healthcare Technologies, Waukesha, WI) was used to image patients in a supine position. From the helical scans, axial slices were reconstructed at 1.25-mm spacing and 1.25-mm thickness for transfer to a workstation for measurement.

The CL/P patients were classified into subgroups: cleft lip or alveolus (CL), unilateral cleft lip and palate (UCLP), bilateral cleft lip and palate (BCLP), and isolated cleft palate (ICP).

Computed tomography data for a relatively normal Malay cohort ($n = 12$) in the same age range were collected during the same time period and made available for this study by Dr. A. Yusof. These patients had presumed normal craniofacial morphology but had indications for CT scanning for problems other than those recognized to cause abnormalities in craniofacial growth and morphology.

Ethical approval was obtained from the Ethics and Research Committee, Universiti Sains Malaysia (USM/PPSG/Ethic Com./2001 [61.3(1)], August 30, 2001).

Measurement

The PERSONA software package (Australian Craniofacial Unit, Adelaide, South Australia) was used for 3D reconstruc-

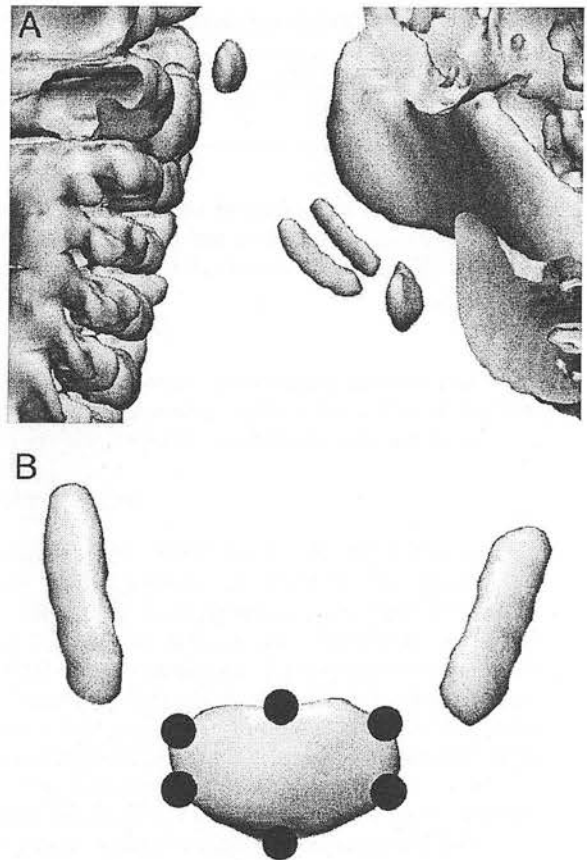


FIGURE 1 A: Right view of a 3D CT reconstruction of the hyoid bone of a normal 4.7-month-old infant showing the body and left and right greater horns. Points used for distance measurements on the right greater horn are shown. B: Anterior close-up view showing points determined on the hyoid body.

tion of the images and to determine the 3D coordinates of osseous landmarks (Abbott et al., 1990, 2000; Netherway et al., 1997, 1999) on a Silicon Graphics Computer workstation (SGI, Mountain View, CA). This package allowed the display of the CT scan data around a 3D marker simultaneously in windows containing axial, sagittal, and coronal reformats and 3D CT reconstructions of the external craniofacial bones and the cranial base. The threshold used for placing landmarks on the bone surface was 150H.

Length and Height of the Greater Horns of the Hyoid Bone

Figure 1 shows the three ossified regions of the normal hyoid bone in a 4.7-month-old infant. Lengths of the greater horns were measured from the posterior tip to the anteroinferior tip, on the right and left sides, representing the inferior length of the hyoid bone. Similarly, the superior length of the hyoid bone was measured from the greater horn's posterior tip to the greater horn's anterosuperior tip on the right and left.

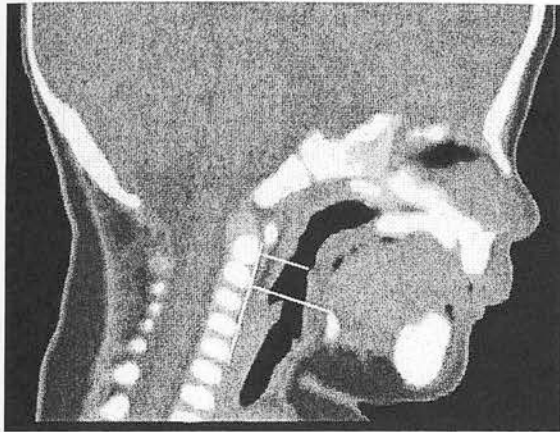


FIGURE 2 Midsagittal CT reformat showing the position of the body of the hyoid bone (opposite lower C2) and tip of the epiglottis (opposite upper C2) for a normal infant.

The anterior height (thickness) of the greater horn was measured from the anteroinferior tip to the anterosuperior tip on the right and left.

Height and Length of the Body of Hyoid Bone

Landmarks used to determine the midline, the left and right heights, and the superior and inferior lengths of the body of the hyoid bone are shown in Figure 1B. These were the six points comprising the inferior and superior, left, right, and midline points on the surface of the body of the hyoid.

Distances From the Hyoid Bone to the Cranial Base

Distances were determined from the most inferior point of the anterior aspect of the foramen magnum in the midsagittal plane (basion) and the most inferior point of the spheno-occipital synchondrosis on the sphenoid bone to the anterior-superior medial surface of the body of the hyoid bone.

Distances of the Hyoid Bone to the Cervical Spine

A reference line was constructed from the most anterior-superior midpoint of C2 to the most anterior-inferior midpoint of C4. The distances from the superior and inferior aspects of the hyoid body to this reference line were then determined from the landmark positions.

Level of the Hyoid Bone and Tip of the Epiglottis Relative to the Cervical Vertebrae

A perpendicular line was drawn from the most superior midline point on the surface of the body of the hyoid bone to a cervical reference line (constructed from C2 to C5, touching the most anterior point on the surface of the bodies of C2 and C5 in the midsagittal plane) to determine the position of the hyoid bone in relation to the cervical vertebrae.

TABLE 1 Age and Sex Distribution of the CL/P and NC Groups

Group	Sex (n)	N	Mean Age (d)	SD	Range
CL/P	F 12 M 17	29	115	76	14-340
NC	F 3 M 9	12	145	86	19-297

A similar line was also drawn from the tip of the epiglottis to the cervical tangent line to determine the position of the epiglottis in relation to the cervical vertebrae (Fig. 2).

Hyoid Bone Angle

The hyoid bone angle was measured between lines drawn from the most superomedial point on the surface of the body of the hyoid to sella and then between sella and nasion.

Statistical Analysis

A linear model (PROC GLM, SAS/STAT User's Guide, Version 8, SAS Institute, Inc., Cary, NC) incorporating the fixed effects of sex (male, female) and cleft group (NC, UCLP, BCLP, ICP, CL) and using age (14 to 340 days) as a covariate was fitted to each measurement. Higher-order interactions were not analyzed for this small data set. Linear contrasts were arranged to compare the control group (NC) with all other groups and to compare the ICP group (a distinct cleft type) with other cleft groups.

Fisher exact test was used to test for associations between anomalies of the hyoid bones and occurrence of CL/P.

Errors of the Method

Repeat determinations were performed for all infants after a period of 1 month to assess the reproducibility of landmark determination and anthropometric variables derived from these landmarks. Systematic errors in landmark location were tested by Hotelling *T* statistic. For anthropometric variables, paired *t* tests were used to detect systematic errors (i.e., to ascertain whether the mean difference between repeated measures deviated significantly from zero), and the Dahlberg (1940) method of double determination was used to quantify the magnitude of random errors.

RESULTS

The age and sex distributions of the CL/P and NC groups are shown in Table 1. The distribution of CL/P patients was as follows: 7 CL, 10 UCLP, 4 BCLP, and 8 ICP. There were 12 patients in the NC group.

The relocation errors for individual landmarks ranged from 0.3 mm for the anteroinferior tip of the left greater horn to 0.5 mm for the left inferior tip of the hyoid body. The pooled landmark relocation error was 0.4 mm. Paired *t* tests between repeat determinations of anthropometric variables indicated that there were systematic errors ($p < .05$) for hyoid to basion distance and for left lower length of the greater horn, right

TABLE 2 Adjusted Means and Standard Errors (SEs) of the Hyoid Bone Measurements

Measurement†	Groups									
	NC (n = 12)		UCLP (n = 10)		BCLP (n = 4)		CL (n = 7)		ICP (n = 8)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
inf length lt GH (mm)*	8.5	0.62	8.8	0.62	10.2	0.96	7.6	0.71	7.0	0.67
inf length rt GH (mm)	8.5	0.56	10.0	0.58	8.3	0.90	7.4	0.67	7.7	0.63
sup length lt GH (mm)*	8.3	0.62	9.0	0.61	9.9	0.95	7.3	0.70	6.8	0.66
sup length rt GH (mm)	8.3	0.56	9.7	0.58	8.4	0.91	7.4	0.67	7.2	0.63
Height lt GH (mm)	2.8	0.16	2.5	0.15	2.4	0.24	2.7	0.18	2.3	0.17
Height rt GH (mm)	2.7	0.16	2.5	0.16	2.2	0.25	2.7	0.18	2.5	0.18
HB height lt (mm)	2.4	0.23	2.2	0.28	2.3	0.45	2.4	0.32	2.4	0.30
HB height rt (mm)	2.2	0.21	2.1	0.25	2.6	0.40	2.3	0.28	2.4	0.27
HB sup length lt (mm)	4.4	0.27	3.9	0.32	4.3	0.52	5.0	0.36	4.1	0.35
HB sup length rt (mm)	4.4	0.24	3.6	0.29	3.8	0.46	4.7	0.32	4.2	0.31
HB inf length lt (mm)	4.1	0.29	3.5	0.35	4.0	0.56	4.2	0.40	4.0	0.38
HB inf length rt (mm)	4.1	0.29	3.5	0.34	3.5	0.55	4.0	0.39	3.4	0.37
HB sup—cervical (mm)	22.1	0.63	20.8	0.72	21.1	1.16	21.3	0.81	19.2	0.77
HB inf—cervical (mm)	22.5	0.64	21.9	0.73	21.6	1.18	22.1	0.83	20.0	0.79
Hyoid—basion (mm)**	27.5	0.72	32.3	0.86	32.2	1.38	29.1	0.97	29.9	0.92
Hyoid—inf SOS (mm)	30.7	0.86	32.3	1.04	33.9	1.68	30.4	1.18	31.9	1.12
Hyoid angle (°)**	92.9	1.76	85.8	2.10	86.2	3.37	88.7	2.37	87.1	2.46

† inf = inferior; lt = left; GH = greater horn; rt = right; sup = superior; HB = hyoid body; SOS = spheno-occipital synchondrosis.

* Significant difference at $p < 0.05$ between ICP and the other cleft groups combined.

** Significant difference at $p < 0.05$ between all cleft groups and the NC group.

upper length of the greater horn, and right height of the hyoid body. However, the mean differences that were found to be statistically significant at the $p < .05$ level were all in the range from 0.1 mm to 0.2 mm, and the random errors, quantified by the Dahlberg statistic, ranged from 0.3 to 0.5 mm. Random errors for other hyoid bone variables ranged from 0.3 mm for height of the left greater horn to 0.7 mm for height of the hyoid body on the left. The error for hyoid bone angle was 1.2° . These measurement errors are approximately one pixel in size (0.488 mm) and indicate that errors in the method were acceptable for this study and unlikely to bias the results.

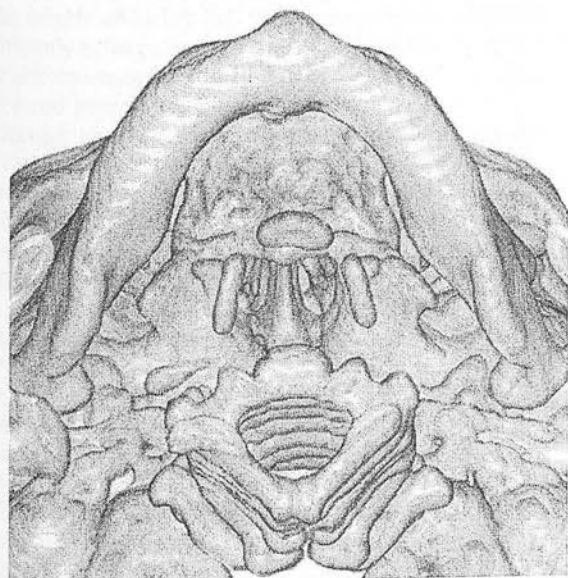


FIGURE 3 Inferior view of a 3D CT reconstruction of the hyoid bone in a normal 4.7-month-old infant.

Table 2 shows adjusted means and their standard errors for the study variables in the four cleft groups and the NC group. No significant differences between CL/P and NC groups were found for left and right lengths of the greater horn. However, the inferior and superior lengths of the left greater horn were significantly smaller in the ICP group compared with other affected groups ($p < .05$) (Figs. 3 and 4).

We examined the data for evidence of a correlation between asymmetry of the greater horns and the side of the cleft in

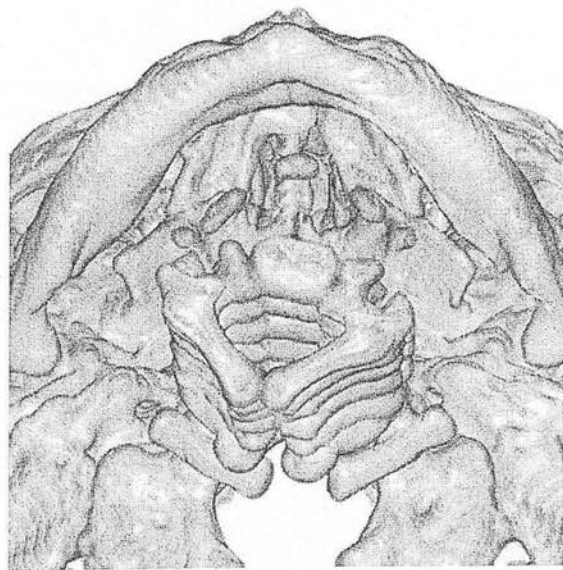


FIGURE 4 Inferior view of a 3D CT reconstruction of the hyoid bone in a 6.8-month-old infant with ICP illustrating the smaller size of the hyoid bone visible as three distinct segments, that is, the central anterior body and the bilateral greater horns.

TABLE 3 Characteristics of the Hyoid Bone and Epiglottis in Malay Infants with CL/P*

Diagnosis	Sex	Age (d)	Hyoid Body Height	Epiglottis Height	Aspiration Pneumonia
CL	F	49	normal	normal	
CL	F	61	normal	normal	
CL	F	77	normal	normal	
CL	M	79	absent	normal	
CL	M	109	normal	low	
CL	M	126	normal	normal	
CL	M	325	low	low	
ICP	F	14	low	low	
ICP	F	82	low	normal	
ICP	F	160	normal	normal	
ICP	F	222	normal	normal	
ICP	F	340	normal	normal	
ICP	M	60	absent	low	
ICP	M	61	low	normal	
ICP	M	206	low	low	yes
UCLP	F	53	normal	normal	
UCLP	F	116	low	low	
UCLP	F	149	low	normal	
UCLP	M	69	normal	low	
UCLP	M	81	absent	normal	yes
UCLP	M	90	low	low	
UCLP	M	92	low	low	
UCLP	M	104	absent	low	
UCLP	M	111	normal	normal	
UCLP	M	141	normal	normal	
BCLP	F	88	low	normal	
BCLP	M	33	absent	low	yes
BCLP	M	94	low	low	
BCLP	M	155	normal	normal	yes

*Normal hyoid body height was taken to be level with mid-C3 and above, whereas normal epiglottis height was taken to be level with mid-C2 and above.

those patients with UCLP. Although 7 of 10 UCLP patients had smaller ipsilateral greater horns, a larger sample size would be required to establish whether there was indeed a tendency for smaller ipsilateral size.

The heights of the left and right greater horns did not differ significantly between the CL/P and NC groups, and no significant differences in the overall size of the body of the hyoid were found between the groups (Table 2).

Although airway distances from the superior and inferior aspects of the hyoid body to the cervical vertebrae for the CL/P group were smaller than for the NC group, this was not significant ($p = .06$). The position of the hyoid bone was significantly lower in relation to the cranial base (basion) in the CL/P group ($p = .0008$). This is consistent with the association tests for position relative to the cervical vertebrae.

The angle between the hyoid bone, sella, and nasion was 4 to 7° smaller in the CL/P group compared with the NC group ($p = .010$). The predominant influence here would be small postural differences between the groups.

For each CL/P individual in the study, Table 3 shows the level of the hyoid body and epiglottis tip relative to the cervical vertebrae, absence of ossification of the hyoid body, and whether the patient had aspiration pneumonia. The hyoid body height was considered normal if the most superior point on the body was opposite mid-C3 or more superior. The epiglottis tip height was considered normal if the tip was opposite mid-

TABLE 4 Association Between CL/P and the Level of the Hyoid Bone ($p = 0.006$)

	CL/P	NC	Total
Mid-C3 and above	13	12	25
Below mid-C3	11	0	11
Total	24	12	36

C2 or more superior. All NC patients had normal hyoid body positions and no record of aspiration pneumonia. Association analysis (Table 4) showed that the hyoid bone was significantly lower (Fisher exact test $p = .006$) in relation to the cervical vertebrae in the CL/P group (Fig. 5).

A similar association test for height of the tip of the epiglottis revealed that the epiglottis was lower for the CL/P patients than for the NC cohort (Fisher exact test $p = .008$). Furthermore, the low position of the hyoid bone was associated (Fisher exact test $p = .033$) with a low epiglottic position.

In the CL/P group, 17% (5/29) of the infants showed no ossification of body of the hyoid bone (Fig. 6), whereas all the NC infants displayed ossification. For the patient shown in Figure 6, the maximum density in the region where the hyoid body would ossify was approximately 50H, well below the surface detection threshold of 150H and substantially lower than the density of the left and right horns at 315H.

The prevalence of aspiration pneumonia within the study group was high, 14% (4/29), relative to the general population. Only one infant studied who had aspiration pneumonia was normal regarding both hyoid body and epiglottis position. Our small sample size precluded finding any significant association between aspiration pneumonia and missing hyoid body, height of the epiglottis, or height of the hyoid (Table 3).

DISCUSSION

To our knowledge, this is the first 3D CT study of the size, morphology, and location of the hyoid bone in unoperated in-

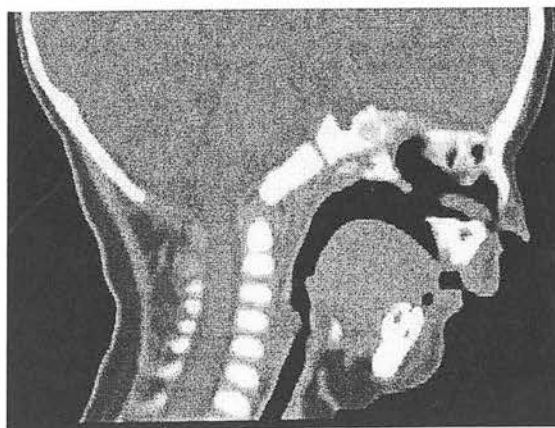


FIGURE 5 Midsagittal CT reformat showing the low level of the hyoid bone (at upper C4) and epiglottis (at lower C2) relative to the cervical vertebrae for a CL/P patient.

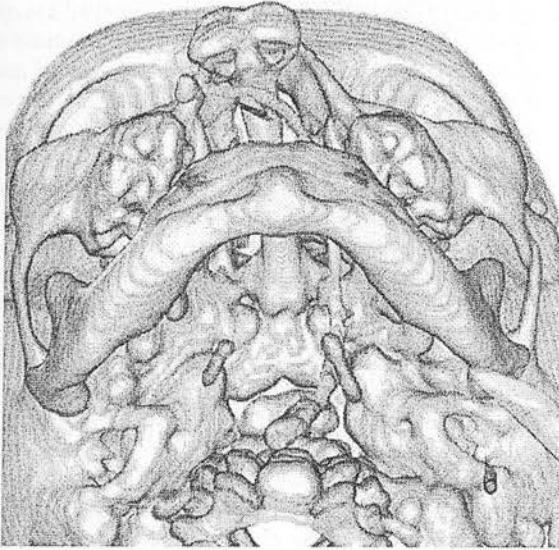


FIGURE 6 Inferior view of a 3D CT reconstruction showing the absence of ossification of the body of the hyoid bone in a 3.4-month-old infant with UCLP.

infants with CL/P. In the NC fetus, at 23 to 25 weeks *in utero*, the fetal hyoid and larynx are situated high relative to the cervical vertebrae, extending from the basiocciput to the level of C3 or C4 (Lieberman et al., 2001). The larynx also tends to be higher in children than in adults, with the hyoid bone found at the level of C2 and C3 from birth to 2 years and at the level of C3 in older children (Hudgins et al., 1997; Liebermann et al., 2001). The latter findings were obtained by CT and magnetic resonance imaging and are consistent with those of an earlier study (Roche and Barkla, 1965) that used conventional radiography.

In the CL/P group, we found that hyoid bone was significantly inferiorly positioned (below mid-C3) compared with the NC group (level with or above mid-C3). One previous study (Kaduk et al., 2003) used lateral cephalometry to compare the position of the hyoid bone in older children with repaired clefts with the position in NC individuals and found that the hyoid bone in CL/P patients was located more inferiorly and anteriorly than in NC subjects. Our results do not support a more anterior location of the hyoid in infants with CL/P.

Although no differences in the lengths of the left and right greater horns of the hyoid bone were found between CL/P and NC groups, the statistical analysis indicated that the length of the left greater horn was smaller in the ICP group compared with the other cleft groups ($p = .035$ and $p = .022$ for the inferior and superior lengths). Although the inferior and superior lengths of the greater horn on the right were smallest for the ICP group, they were not significantly so. A larger sample size would resolve whether the greater horns were indeed smaller for ICP than for other CL/P groups. We speculate that a difference may result from the different etiology of ICP compared with the other CLP groups.

In 17% (5/29) of the CL/P infants, there was no evidence of ossification of the body of the hyoid bone (Fig. 6). All CL/P groups were affected (two for UCLP and one each for BCLP, ICP, and CL). The hyoid body was visible for each patient in the NC group. This finding suggests a high prevalence of delayed ossification of the hyoid body in CL/P patients compared with NC individuals. The normal age range for radiographically visible ossification of the hyoid body is not known, though Wells et al. (1986) found that 75% of autopsied control neonates (younger than 1 postnatal month) had a radiographically visible hyoid body. The youngest patient in our series with nonvisible ossification of the hyoid body was 33 days old (Table 3). The rest of the patients with a nonvisible hyoid body were substantially older (2 to 3 months) than the patients in the study of Wells et al. (1986). If delayed ossification is associated with CL/P, this would provide further evidence that during embryological development the underlying factors associated with clefting not only affect the labiomaxillary and palatine structures of the first arch, but also influence the development of structures derived from the second and third branchial arches. There is also the possibility that delay in ossification reflects poor health or nutrition, that is, a secondary rather than a primary effect of the cleft.

The anomalies of the hyoid bone noted in this study are consistent with an alteration in ossification pattern or skeletal development. We do not know whether these findings merely represent a developmental delay in hyoid bone development, so this hypothesis remains as the premise for further study of hyoid bone development in children with CL/P.

Anomalies of the hyoid bone may contribute to abnormal swallowing and breathing patterns in some infants. Previous studies have shown that infants need to exert additional force to open a closed pharyngeal airway compared with that required to maintain its patency, presumably because of airway wall adhesion (Wilson et al., 1980, 1981). For CL/P infants with abnormal ossification or position of the hyoid bone, the functions of swallowing and breathing may be compromised. Consequentially, an incomplete epiglottal seal could lead to laryngeal penetration by aspiration. This situation represents inefficiency in the proper coordination of swallowing and breathing and may predispose certain infants to the aspiration of pharyngeal contents.

As could be expected, the low position of the hyoid bone was associated with a lower epiglottis position (Fig. 5). Sasaki et al. (1977) reported on postnatal descent of the epiglottis and concluded that the age interval from 4 to 6 months seems to represent a transitional period from obligatory nasal breathing to potential oral respiration. The age of infants in the current study includes this time period. Moreover, our observations of delayed ossification of the hyoid and premature descent of the hyoid bone and epiglottis suggest that there may be an association with breathing disorders in CL/P infants.

It is possible that an association may exist between these hyoid anomalies and the problems that CL/P infants have with suckling, swallowing, and the propensity for chest infection. Bamford et al. (1992) showed that normal newborns have a

stable and uniform swallow rate within 48 hours of birth, but their breathing is not coordinated with swallowing, with ventilation being reduced during feeding. From that study, it appears that mild and transient oxygen desaturation (hypoventilation) is well tolerated by newborns, but the same may not be true for older infants with an increasing metabolic demand, a decreased tolerance for hypoxia, and an increased respiratory drive. This study indicates that infants with CL/P have aspiration pneumonia quite commonly (14%) in the newborn-to-6-months age range. This respiratory illness occurred in our sample in three of four patients with BCLP and one of eight patients with ICP at around 4 months of age. Although our CL/P group had a high prevalence of aspiration pneumonia, a direct association with hyoid position or body absence was not established by our study.

Azmi et al. (1983) found an association between the occurrence of CL/P, esophageal atresia, and tracheo-esophageal fistula. They suggested that this association could cause clinical problems, such as difficulties in swallowing and recurrent chest infections. In addition, they recommended that the possible presence of second and further hidden anomalies should be fully investigated in a newborn with obvious craniofacial malformations, and that delays in diagnosis should be minimized. In our study, there was clinical suspicion that two of the infants may have had tracheo-esophageal fistulae, requiring investigation with CT.

The findings of this study have opened several avenues of future research. The application of geometric morphometric analyses to the landmark data would enable a more detailed assessment of whether the hyoid is altered in shape in CL/P infants. A more extensive study based on larger numbers also seems justified to determine the prevalence of aspiration pneumonia and tracheo-esophageal fistula in CL/P infants. More reliable records of the incidence of aspiration pneumonia would be desirable so that the suggestion of a relationship between anatomical variations of the hyoid bone and functional problems relating to breathing and swallowing could be confirmed. Finally, because this study was conducted with Malays, future studies are required to ascertain whether similar variations in location and morphology of the hyoid bone in CL/P also occur in other ethnic groups.

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A Three-Dimensional Computed Tomography Analysis of Craniofacial Asymmetry in Malaysian Infants with Cleft Lip and Palate

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Abstract

Background: The application of three-dimensional computed tomography (3D CT) to analyse craniofacial morphology in individuals with cleft lip and palate (CLP) enables detailed assessments to be made of asymmetry in the region of the cleft and in regions distant from the cleft. The aim of this study was to compare craniofacial morphology in a sample of Malaysian infants with unoperated CLP with a control sample of unaffected Malaysian infants.

Methods: The study sample comprised 29 individuals: 10 with unilateral CLP (UCLP), 5 with bilateral CLP (BCLP), 7 with cleft lip and primary palate (CLPP), and 7 with isolated cleft palate (ICP). The control sample consisted of 12 non-cleft (NC) infants. All subjects were between 0.4 and 12.2 months of age. Nine mid-facial and 4 nasal bone landmarks were located on 3D CT scans and compared to a midline reference plane, which was created using the landmarks basion, sella, and nasion. Unpaired *t* tests and *F* tests were used to compare means and variances between sample groups, whereas paired *t* tests were used for comparisons within the UCLP and NC groups.

Results: Differences in variances of some mid-facial breadths and nasal bone dimensions were found in both male and female cleft groups when compared to the NC sample. In the UCLP group, some nasal bone and facial breadth dimensions were larger than in the NC sample and the nasal bone tended to deviate to the contralateral side of the cleft.

Conclusion: CLP affects the size and orientation of the nasal bones and is associated with an altered morphology of some facial bones at positions distant from the region of the cleft.

Keywords: cleft lip, cleft palate, facial asymmetry, infant, radiology, three-dimensional imaging, tomography

Introduction

Patients with orofacial clefts present with a variety of problems including dental anomalies, malocclusions, disorders of speech and hearing, and secondary facial deformities (1,2). Non-syndromic cleft lip, with or without cleft palate, is relatively common. It demonstrates a prevalence that ranges from 0.04 to 0.79 per 1000 live births (3) and 1 in every 500 to 550 live births, with the highest rates observed among the Asians (4). Although functional or iatrogenic factors are generally thought to affect normal facial morphology and growth potential (5,6), it is understood that there is an underlying genetic basis for the formation of clefts (7). Specifically, the *MSX1* gene has been associated with cleft

palate, and the *MSX1* and *TGFβ3* genes have been associated with cleft lip, with or without cleft palate (7,8). Conversely, other researchers have found little evidence supporting these findings (9). Changes in facial growth and development in cleft children likely reflect the combined effect of genes and the environment; that is, clefts result from multifactorial influences that affect the growth potential of the face and the overall symmetry of the soft tissues and facial bones (5). Regardless of the pathogenesis or genetics, anomalous developmental conditions, such as cleft lip and palate (CLP), are often associated with increased levels of asymmetry, which have been described as fluctuating or directional asymmetry (10). Fluctuating asymmetry refers to small, random differences in size between sides

of the body, for example the face, and is thought to reflect developmental instability, whereas directional asymmetry involves a consistent trend in which one side is larger or smaller than the other and may be influenced by homeobox genes (10–12). The assessment of facial asymmetry is an important component of evaluating the success of surgical repair in CLP and is linked to psychological issues such as perceptions of attractiveness and intelligence (13). Therefore, the present study included an assessment of asymmetry by comparing landmark measurements from the left and right sides of the face.

Methodologically, cephalometric and panoramic radiographs have traditionally served as the primary option for the radiographic analysis of craniofacial morphology. However, there are limitations in the measurement of asymmetry using two-dimensional (2D) radiographs, such as the super-imposition of structures and the reliance on machine positioning relative to the external auditory meati, which can be asymmetric within individuals (14). Hence, three dimensional (3D) imaging techniques have been developed to overcome the shortcomings of conventional 2D methods and were applied in the present study; specifically, 3D computed tomography (CT) was used. Other available 3D imaging techniques include morphoanalysis, laser scanning, stereolithography, 3D ultrasonography, 3D facial morphometry, digigraph imaging, Moiré topography, and contour photography (1). Data obtained with 3D CT scanning can be used for soft and hard tissues analysis, whereas methods based on laser techniques are used mainly for the analysis of soft tissue surfaces. Consequently, 3D CT scanning was deemed most suitable for data collection in our study.

The overall aim of this study was to compare the craniofacial morphologies in a sample of unoperated Malaysian infants with CLP with those in a sample of age-matched, unaffected, non-cleft (NC) Malaysian infants. Differences in morphologies of the nasal bones were emphasised. A midline plane constructed from the landmarks basion (ba), sella (s), and nasion (n) was used to assess asymmetry in the selected craniofacial variables in both the CLP and the NC groups.

Materials and Methods

The Malaysian patient database established at the Australian Craniofacial Unit (ACFU), Adelaide Women's and Children's Hospital, provided the 3D CT scans of the subjects. The

Malaysian cleft sample comprised 29 randomly selected individuals (12 females, 17 males): 10 with unilateral CLP (UCLP), 5 with bilateral CLP (BCLP), 7 with cleft lip and primary palate (CLPP), and 7 with isolated cleft palate (ICP). The control (NC) sample consisted of 12 Malaysian infants (4 females, 8 males) with no craniofacial abnormalities. Ideally, CT scans obtained from normal individuals would provide the ideal control group; however, the radiation dose involved in acquiring CT scans of healthy individuals cannot be justified. There should be sufficient medical and diagnostic reasons for performing a CT investigation. Hence, the NC subjects in the present study were patients with medical conditions that were sufficiently significant to justify the performance of CT scans (for example, meningitis and mild hydrocephalus). However, these conditions did not cause abnormalities in craniofacial growth and morphology (15), as confirmed by preliminary comparisons of the cranial base and facial dimensions of individuals with mild hydrocephalus and of other controls, which revealed estimates within the normal measurement range. All individuals included in the study were of Malay ethnicity. The age of the cleft patients ranged 1.1–12.2 months with a mean of 3.8 (SD 2.5) months, whereas the age for the NC group ranged 0.4–11.9 months with a mean of 4.8 (SD 2.8) months. Ethical approval was obtained from the Adelaide Women's and Children's Hospital Research Ethics Committee.

The Persona software package developed at the ACFU was utilised for 3D reconstruction of the craniofacial images and determination of the 3D coordinates of osseous landmarks on a silicon graphics computer workstation. This package enables the display of the CT scan data simultaneously around a 3D marker in windows showing axial, sagittal, and coronal sections, and it provides an accurate 3D reconstruction of the external craniofacial bones and the cranial base. The Persona software package enables the 3D positions of landmarks to be located with high precision, which allows the automatic generation of slices through selected points. The thickness of the scan data slices can vary 1.25–2.00 mm. Preliminary analyses using 68 landmark comparisons (61 distances, 7 angles) indicated the presence of random measurement errors ranging 0.2–1.1 mm for distances between landmarks, whereas the random errors for angular variables ranged 1.0°–2.7° (15). In general, the measurement errors were considered relatively small and unlikely to bias the results.

In the present study, 13 osseous landmarks were located on the mid-facial region of subjects due to their close proximity to the clefts (Table 1, Figure 1) (16,17). A midline reference plane was created using the following landmarks: ba, s, and n (Figure 2). Breadth variables were then estimated by measuring the distances from mid-face osseous landmarks to the midline reference plane, whereas nasal bone dimensions were estimated by measuring the distances and angles between nasal osseous landmarks (Figure 3).

The influence of gender was investigated by comparing variables between male and female subjects in both cleft and NC samples. To explore the presence of any association between the side of the cleft and the direction of nasal bone deviations, the UCLP and NC samples were compared as follows: bilateral variables that coincided with the location of the cleft were measured, and asymmetry was assessed by subtracting the ipsilateral from the contralateral measurements.

The data were screened and subsequently corrected for outliers when necessary. Double determinations were performed to assess the magnitude of any systematic or random errors, and Dahlberg statistics were calculated for each variable (18).

Although the 2 groups were as closely matched for age as possible, the age range in the cleft group was slightly greater than that in the NC group. Additional age adjustments were applied to the data following the regression analyses of each variable against age in both the cleft and NC samples. Hence, all of the presented data are age-adjusted.

Comparison of the mean values and variances between male and female cleft and NC groups were performed using unpaired t tests and F tests with a significance level of $P < 0.05$. Comparisons between measurements on right and left sides of the face within the UCLP group and within the NC sample were conducted using paired t tests. The R (R Foundation for Statistical Computing, Vienna, AT) and Excel (Microsoft Corporation, Redmond, WA, US) statistical programmes were used to analyse the collected data.

Results

Male cleft and NC samples

Table 2 shows selected landmark distances which revealed the greatest differences in mean values between male cleft and NC samples. Cleft males exhibited greater distances from mid-face

landmarks (snml, orl, gol, ztl, ztr, and ofl) to the midline reference plane (na-s-ba) and greater breadth distances (ofl-ofr, gol-gor, and ztl-ztr) than did NC males but none of these differences in mean values was significant statistically. However, 5 of the variables (gol, ztl, ztr, gol-gor, and ztl-ztr) displayed significantly unequal variances ($P < 0.05$), with variances in NC males exceeding those in cleft males.

Female cleft and NC samples

Table 3 presents selected landmark distances and angles which showed the greatest differences in mean values between female cleft and NC samples. All variables were larger in the female cleft group than the NC group, including the distances from mid-face landmarks to the midline plane (inmr, eul, gor, mal, pol, and ztr) and the mid-facial breadths (mal-mar). Dimensions of the nasal bone that showed the largest differences between the female cleft group compared with the NC group were na-n and inmr. Angulations depicted by the variables snml-n-snmr and inml-na-inmr were also larger in the female cleft group compared with the NC group. None of these differences in mean values was significant statistically, although the difference in mean values for inmr to the midline plane and snml-n-snmr both reached borderline significance ($P = 0.05$). Five of the variables (eul, mal, ztr, mal-mar, and n-na plane) showed significant heterogeneity in their variances ($P < 0.05$ each), with variances in NC females exceeding those in cleft females for all variables except n-na plane. The nasal bone in the female cleft group showed a significant deviation to the right compared with the NC sample ($P < 0.05$) and this angle also showed significantly greater variability in the cleft group compared with the NC group ($P < 0.05$).

UCLP and NC samples

Comparison of the UCLP and NC samples (prior to consideration of the cleft location) revealed several statistically significant differences in mean landmark distances, as reported in Table 4. All nasal bone dimensions were larger in the UCLP group than the NC group, with significant differences in dimensions na-n, snmr-n, and snml-snmr ($P < 0.05$ each). Facial breadth distances were also larger in the UCLP group than the NC group, with significant differences in dimensions orl-orr and ztl-ztr ($P < 0.05$ each).

Table 5 presents the bilateral variables that were associated with significant differences

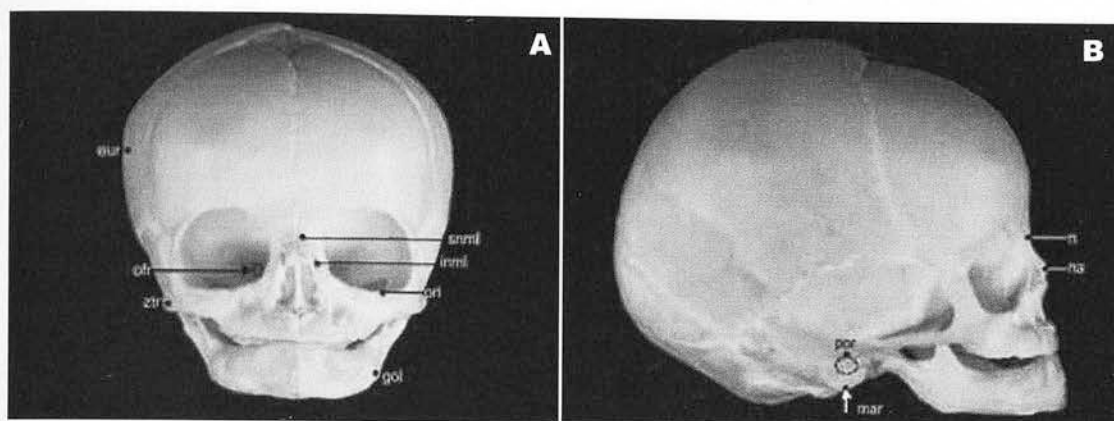


Figure 1: (A) Frontal and (B) right lateral views of a skull depicting osseous landmarks described in Table 1 (excluding sella and basion)

Table 1: Description of the 13 osseous landmarks identified on three-dimensional computed tomography scans (16,17)

Landmark	Abbreviation	Definition
Nasale	na	Tip of the nasal bone
Superior naso-maxillare l/r	snml/snmr	Most superior point on the naso-maxillary suture
Inferior naso-maxillare l/r	inml/inmr	Most inferior point on the naso-maxillary suture
Nasion	n	Most anterior point on the fronto-nasal suture (when the suture was not clearly identifiable, the deepest point on the nasal notch was substituted)
Sella	s	Centre of the sella turcica
Basion	ba	Mid-sagittal point on the anterior margin of the foramen magnum (at the saddle point)
Gonion l/r	gol/gor	Point on the angle of the mandible located by bisection of the angle formed by the mandibular line and the ramus line
Orbitale l/r	orl/orr	Most inferior point on the infraorbital margin
Zygo-temporale l/r	ztl/ztr	Mid-point of the bony concavity formed between the frontal and temporal processes of the zygomatic bone
Optic foramen l/r	ofl/ofr	Centre of the anterior opening of the optic canal
Porion l/r	pol/por	Most superior point on the margin of the external auditory meatus
Mastoidale l/r	mal/mar	Most inferior point on the mastoid process
Euryon l/r	eul/eur	Most lateral point on the skull

Letters l and r denote left and right, respectively.

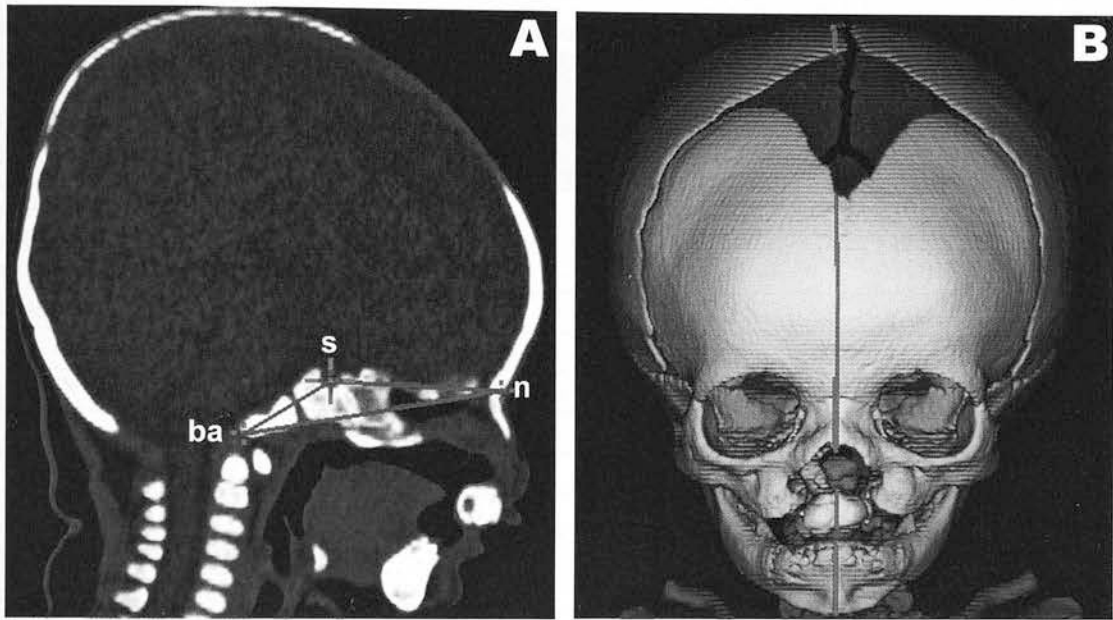


Figure 2: Computed tomography images of the reference plane constructed from 3 osseous landmarks: nasion (n), sella (s), and basion (ba). (A) Sagittal view of “wire frame” constructed midline reference plane. (B) Frontal view of reference plane bisecting a BCLP patient.

between right and left sides of the face when the location of the cleft was considered. To determine cleft-side (ipsilateral) to non-cleft-side (contralateral) dimensional differences, measurements for the ipsilateral side of the cleft were subtracted from those obtained for the contralateral side of the cleft. The results showed that the distances from the ipsilateral zt and the contralateral zt to the midline reference plane were significantly different ($P < 0.05$), with a larger distance measured on the contralateral side of the cleft. Additionally, a significant degree of deviation ($P < 0.001$) was observed for the nasal bone variable na–n, which deviated away from the cleft side. In the NC sample, no significant differences were detected between the left and right sides of the face.

Discussion

Despite several growth theories (19), our understanding of the cellular and molecular control mechanisms involved in human craniofacial development remains incomplete. It is believed that during the course of normal craniofacial development, the histogenesis and functional maturity of muscles, nerves, and vessels may influence one another (19). Abnormal craniofacial development, such as clefting, is also likely to influence the growth and development

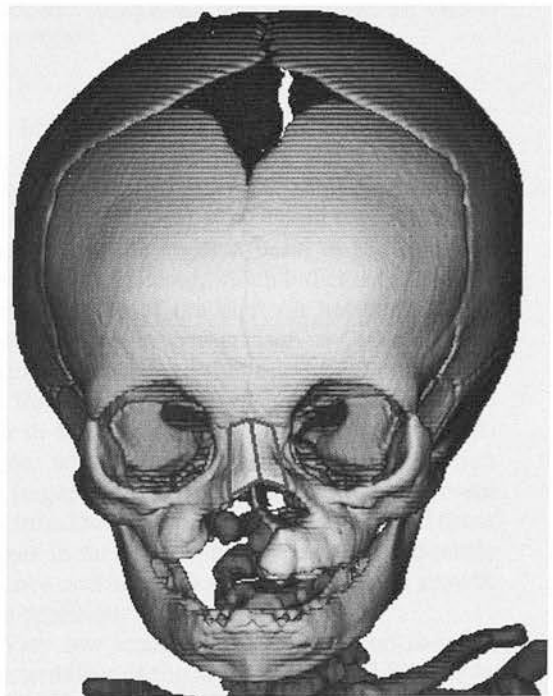


Figure 3: Frontal view of three-dimensional computed tomography image of nasal bone “wire frame”.

Table 2: Descriptive statistics for selected variables in male non-cleft (NC) and cleft groups

	Males NC (n = 8)			Males Cleft (n = 17)			
	Mean (mm)	SEM	SD	Mean (mm)	SEM	SD	P value
Distance from landmark to midline plane, na-s-ba (mm)							
Snml (nasal bone)	- 3.64	0.33	0.93	- 4.52	0.28	1.15	NS
Orl	- 16.81	0.64	1.81	- 18.01	0.37	1.53	NS
Gol	- 26.59	1.37	3.87	- 28.52	0.56	2.31	0.04*
Ztl	- 37.15	1.72	4.86	- 39.32	0.63	2.60	0.02*
Ztr	37.44	1.77	5.01	39.47	0.64	2.64	0.02*
Ofl	- 7.90	0.42	1.19	- 8.77	0.23	0.95	NS
Breadth distance (mm)							
Ofl-ofr	16.05	0.72	2.04	17.24	0.43	1.77	NS
Gol-gor	53.05	2.51	7.10	56.58	0.97	4.00	0.03*
Ztl-ztr	74.64	3.49	9.87	78.94	1.24	5.11	0.01*

Positive mean values indicate the right side of the skull, while negative mean values indicate the left side of the skull. * $P < 0.05$ indicates significant difference and NS indicates non-significant difference ($P > 0.05$) in variances between NC and cleft groups by *F* test.

Abbreviations: gol = gonion left, gor = gonion right, ofl = optic foramen left, ofr = optic foramen right, orl = orbitale left, snml = superior naso-maxillare left, ztl = zygo-temporale left, ztr = zygo-temporale right.

of adjacent facial and dental structures, which can result in noticeable alterations in facial shape and symmetry. By comparing landmark variables between cleft and NC individuals by gender, it was possible to explore the dimensional impact of clefting on the adjacent facial structures and to assess whether clefting affects males and females differently. Hence, our findings provide information that is important for practising dentists, who play an important role within the multidisciplinary team of health professionals that manage cleft patients.

Earlier research on sex differences in CLP has demonstrated little variation in the craniofacial morphology of infants (20) or children aged 6 to 10 years (21,22). Our study demonstrated that mean measurements tended to be larger in the cleft sample than the NC sample, for both males and females. There were also some variables that showed significant heterogeneity in variances between cleft and NC samples for both sexes. Mid-facial breadths in the combined cleft sample revealed that an orofacial cleft may influence facial growth away from the immediate cleft location and contribute to asymmetry. Asymmetry in cleft patients has been reported in the orbital, maxillary and nasal regions (23). Similar results

were obtained in the present study; the left optic foramen and orbitale in the male sample tended to be further from the midline compared with the contralateral side. There was also some evidence that the zygoma bone in the mid-face region may be affected, which indicates a possible direct influence of the cleft on horizontal mid-facial breadths in comparison with unaffected individuals. We found that regardless of the cleft type, the mandible showed some tendency to be larger in the cleft sample compared with the NC sample, which differs from previous research (24). In general, clefts can influence facial growth away from the immediate cleft location, and these changes in facial morphology may subsequently influence oral function and alignment and growth of the dentition.

Very few studies have reported on the size and orientation of the nasal bones in CLP patients using either radiographs (25) or 3D CT (26), and to our knowledge, no studies have provided results concerning asymmetry. We found that males with clefts tended to show larger superior portions of the left nasal bone and greater left mid-facial breadths compared with the NC group, which suggested potentially left-dominant facial growth. In females, clefts had a somewhat

different effect on nasal bone morphology, which tended to be larger superiorly and deviated to the right with a flatter and longer shape. This morphology suggested a possible effect of the cleft on the prominence of the nasal bridge. Results reported in the literature investigating nasal bone morphology range from reports of considerably shorter nasal bones in subjects with cleft lip compared with subjects with cleft palate (25), to longer nasal bones in cleft patients from 6 years of age through to adulthood compared with non-cleft individuals (26). A combination of the cleft location together with normal lateral growth of the frontal bone and maxilla may explain the increase in nasal bone angulation observed superiorly. It is possible that the inferior dimensions of the nasal bone are less affected by CLP because they form the superior portion of the nasal cavity and are therefore influenced to a lesser degree by the surrounding craniofacial bones. Furthermore,

the facial muscles may affect the growth and deviation of facial bones, including the nasal bones (19). It is important to bear in mind that there are differences in craniofacial morphology among ethnic groups and caution is needed in extrapolating findings from one population to another. Nevertheless, head breadth dimensions in Malaysian infants in the 0–1 age group are similar to those reported for Caucasians (15).

Analysis of the UCLP group showed that severe clefts together with dominant lateral growth of the skull resulted in a number of significant differences between the UCLP and the NC groups. These findings are supported by previous research with respect to transverse asymmetry in individuals with UCLP (27,28,29). Nasal bone lengths in UCLP tended to be longer in both vertical and horizontal dimensions compared with the NC group. This result is supported by evidence showing that UCLP individuals have

Table 3: Descriptive statistics for selected variables in female non-cleft (NC) and cleft groups

	Females NC (<i>n</i> = 4)			Females Cleft (<i>n</i> = 12)			
	Mean (mm/°)	SEM	SD	Mean (mm/°)	SEM	SD	P value
Distance from landmark to midline plane, na--s--ba (mm)							
Inmr (nasal bone)	4.58	0.42	0.84	5.86	0.37	1.28	NS
Eur	- 51.43	4.57	9.14	- 54.66	1.31	4.54	0.04*
Gor	24.30	1.81	3.62	25.86	0.66	2.29	NS
Mal	- 30.18	3.57	7.14	- 32.40	0.84	2.91	0.01*
Pol	- 31.35	2.42	4.84	- 33.24	0.82	2.84	NS
Ztr	34.20	2.87	5.74	36.47	0.81	2.81	0.03*
Breadth distance (mm)							
Mal--mar	59.83	6.46	12.92	63.42	1.60	5.54	0.02*
Nasal bone distance (mm)							
Na--n	10.03	1.46	2.92	11.41	0.47	1.63	NS
Angle (°)							
Snml--n--snmr	123.03	5.86	11.36	140.50	4.50	15.59	NS
Inml--na--inmr	107.10	7.39	14.78	120.58	2.58	9.80	NS
Nasal bone deviation (°)							
n--na plane	- 4.00	0.65	1.30	2.00	1.52	5.27	<0.001**

Positive mean values indicate the right side of the skull, while negative mean values indicate the left side of the skull. **P* < 0.05 indicates significant difference, ***P* < 0.001 indicates highly significant difference, and NS indicates non-significant difference (*P* > 0.05) in variances between NC and cleft groups by *F* test.

Abbreviations: eul = euryon left, gol = gonion left, gor = gonion right, inml = inferior naso-maxillare left, inmr = inferior naso-maxillare right, mal = mastoidale left, mar = mastoidale right, n = nasion, na = nasale, pol = porion left, snml = superior naso-maxillare left, snmr = superior naso-maxillare right, ztr = zygo-temporale right.

a high frequency of disproportionately wide noses in relation to the nose height both pre- and post-surgical treatment (30), whereas other researchers have documented that children with UCLP have significant nasal asymmetry that persists after primary surgery (13). In the present study, a significant degree of nasal bone deviation away from the cleft was detected in the UCLP group.

The 3D CT technology employed in this study provides more accurate and reliable measurements

compared with earlier methodologies that utilise coronal cephalometric or panoramic radiographs. These methods are limited due to super-imposition; for example, landmarks that are positioned more posteriorly, such as s and ba, may be difficult to locate due to overlap with more anteriorly positioned anatomical structures. Furthermore, cephalometric results rely on positioning the radiographic unit relative to the external auditory meati, which can exhibit intra- and inter-individual variations. Although there

Table 4: Descriptive statistics for nasal bone and facial breadth dimensions in cleft lip and palate (UCLP), without considering the side of the cleft, and non-cleft (NC) control groups

	UCLP (n = 10)			NC (n = 12)			P value
	Mean (mm)	SEM	SD	Mean (mm)	SEM	SD	
Nasal bone distance (mm)							
Na--n	12.58	0.44	1.39	10.66	0.58	2.01	0.02*
Inml--snml	13.43	0.51	1.61	11.70	0.61	2.11	NS
Inmr--snmr	13.27	0.50	1.58	11.90	0.57	1.97	NS
Snml--n	5.52	0.46	1.45	4.48	0.38	1.32	NS
Snmr--n	5.61	0.48	1.52	4.33	0.33	1.14	0.03*
Snml--snmr	10.50	0.91	2.88	7.79	0.62	2.15	0.02*
Breadth distance (mm)							
Orl--orr	36.72	1.24	3.92	33.28	0.94	3.26	0.04*
Gol--gor	57.11	1.40	4.43	52.09	2.11	7.31	NS
Mal--mar	68.85	1.28	4.05	63.93	3.19	11.05	NS
Ztl--ztr	80.49	1.60	5.06	72.87	3.01	10.43	0.04*

*P < 0.05 indicates significant difference and NS indicates non-significant difference (P > 0.05) in mean values between NC and UCLP groups by unpaired t test.

Abbreviations: gol = gonion left, gor = gonion right, inml = inferior naso-maxillare left, inmr = inferior naso-maxillare right, mal = mastoidale left, mar = mastoidale right, n = nasion, na = nasale, orl = orbitale left, orr = orbitale right, snml = superior naso-maxillare left, snmr = superior naso-maxillare right, ztl = zygo-temporale left, ztr = zygo-temporale right.

Table 5: Mean distances that demonstrated statistically significant differences between ipsilateral and contralateral sides in the UCLP group (n = 10)

	Mean (mm)	SEM	SD	P value
Nasal bone distance (mm)				
Na-n	-7.97	0.88	2.78	<0.001*
Mid-facelandmark distances (mm)				
Zt-zt	0.72	0.31	0.98	0.045*

Positive mean value indicates the ipsilateral side of the cleft, while negative mean value indicates the contralateral side of the cleft. *P < 0.05 indicates significant difference and **P < 0.001 indicates highly significant difference in mean values between ipsilateral and contralateral sides in the UCLP group by paired t test.

Abbreviations: n = nasion, na = nasale, zt = zygo-temporale.

are advantages in using the 3D CT methodology, technological advances lead to the loss of some comparability between studies with software updates, e.g., differences in the definitions and identification of landmarks between different software programs.

A relatively small sample size and pooling of the different types of clefts for some of the analyses present further limitations to the present study. However, considering the difficulties involved in obtaining samples from unoperated CLP patients for whom CT scans are available, we think that the sample size is acceptable. It is expected that infants with an isolated cleft palate ($n = 7$) are more likely to demonstrate facial morphologies that are more symmetric than those of infants with UCLP. Hence, analyses that explored differences in facial asymmetry between cleft and NC groups (Tables 4 and 5) were not based on pooled cleft data and included only UCLP infants from the cleft group. Given that the aims of the present study were to compare craniofacial morphology, including asymmetry, between samples of unoperated infants with CLP (regardless of the cleft type) and a control NC sample of unaffected infants, we consider the aims to have been adequately met by the pooling of cleft types for some but not all of the presented analyses. An additional issue related to the sample is the age distribution in the cleft and NC groups. The age range in the cleft group was slightly greater, i.e., 1.1–12.2 months and a mean of 3.8 (SD 2.5) months, than that in the NC group, i.e., 0.4–11.9 months and a mean of 4.8 (SD 2.8) months. A few older children were included in the cleft group because their primary operation had been postponed due to other health problems, such as upper respiratory tract infection and aspiration pneumonia. Although this represents a limitation of the present study, the cleft and NC groups were age-matched as closely as possible. They demonstrated very similar age distributions, means, and SDs, and all of the presented data were age-adjusted.

Our assumption that the midline points ($n-s-ba$) can reliably represent a mid-facial plane that divides the face into two equal halves has been drawn from the literature (27,31); however, this may be debatable. For example, the spatial position of n could be affected by the type of cleft, and those of ba and s may be affected in subjects with hydrocephalus. Therefore, preliminary analyses were conducted and revealed that the positions of the 3 landmarks were apparently not significantly affected by abnormalities in craniofacial morphology in either the cleft or NC groups. A number of investigators have reported

significant differences in the size and shape of the cranial base of individuals with CLP compared with NC individuals. In contrast, very few differences in post-natal cranial base morphology and growth have been noted between individuals with isolated cleft lip and NC individuals. In the present study, the cranial base values ($n-ba$ and $n-s-ba$) did not differ significantly between the groups. Although not demonstrated in our study, a trend toward a greater cranial base length in NC individuals compared to cleft individuals has been reported previously (32).

Conclusion

Differences in mid-facial breadths and nasal bone dimensions were associated with clefting (UCLP versus NC). The nasal bones of individuals in the UCLP group deviated away from the cleft. It is important for members of the multidisciplinary team that manages cleft patients to have an understanding of how clefts affect not only dental and oral structures but also other surrounding anatomical structures. This study shows that CLP affects the size and orientation of the nasal bones and is also associated with alterations in the morphology of other facial bones at positions distant from the region of the cleft.

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Authors' contributions

Conception and design: NT, ZR, AY, GT
 Analysis and interpretation of the data: NT, SM, GT
 Drafting of the article: NT, SM, PJA, GT
 Critical revision of the article: SM, PJA, GT
 Final approval of the article: NT, SM, ZR, AY, PJA, GT
 Provision of patients, collection and assembly of data: ZR, AY
 Statistical expertise: GT
 Obtaining of funding: NT, GT

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Intracranial volume of patients with nonsyndromal craniosynostosis

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Object. In recent years, comparisons between intracranial volumes (ICVs) of patients with craniosynostosis and healthy patients have given variable results, leading to questions regarding the validity of the normal reference material and the comparability of the measurement techniques. In this study, ICVs of patients with nonsyndromal craniosynostosis without previous surgical intervention were compared with the ICVs of a normal population of European descent determined using the same method for each group.

Methods. Determination of ICV was based on measuring the area of intersection in each computerized tomography slice. For comparisons the ICV measurements for each patient were standardized with regard to age and sex by expressing them in terms of the standard deviation score.

Only the group of boys with metopic synostosis had a tendency toward smaller ICV than did healthy boys ($p = 0.04$). Partitioning the male metopic data into age groups younger and older than 7 months of age revealed that the younger children had normal ICVs, whereas the older children had, on average, smaller ICVs ($p = 0.02$). Both the female sagittal synostosis and the male unilateral coronal synostosis groups had larger than normal ICVs, both with a probability value less than 0.001.

Conclusions. No evidence was found that the ICVs of patients with nonsyndromal craniosynostosis are smaller than those of normal children, except for boys older than 7 months of age with metopic synostosis. This finding may have implications for the timing of surgical intervention for these patients. The indications are that interventions should be focused less on ICV and more on normalizing craniofacial shape and promoting normal development.

KEY WORDS • intracranial volume • craniosynostosis • computerized tomography • pediatric neurosurgery

PATIENTS with nonsyndromal craniosynostosis have a recognizable cranial deformity named after the involved sutures, although descriptions based on head shape are commonly used (such as scaphocephaly, trigonocephaly, frontal synostotic plagiocephaly, brachycephaly and synostotic occipital [or posterior] plagiocephaly for sagittal, metopic, unilateral coronal, bilateral coronal, and unilateral lambdoid synostosis, respectively).³ Usually sporadic, nonsyndromal craniosynostosis is the most common form of craniosynostosis.

Gault and colleagues,⁴ using CT determinations of ICV and Lichtenberg's⁵ cephalometric determination of normal ICV, investigated whether craniosynostosis results in a smaller-than-normal ICV. They found that most individuals were in the normal range, although the ICVs for all craniosynostosis groups, other than for Apert syndrome, were

smaller than normal. They found that the SD scores were statistically significant only if all data were pooled and patients with Apert syndrome were excluded. Posnick and colleagues¹⁰ measured preoperative ICVs for patients with metopic and sagittal synostosis and found that their children's preoperative ICVs were generally above the age- and sex-matched norms of Lichtenberg. Comments on that paper raised questions regarding the comparative normal data and the methods for obtaining them.⁵ Netherway and colleagues⁸ found that the ICVs of patients with nonsyndromal craniosynostosis were not smaller than those found in the Abbott-Netherway¹ CT-determined normative data. With a larger sample size, Anderson and colleagues² reported reduced ICVs for patients with metopic synostosis.

In this paper, the ICVs of patients with a range of nonsyndromal craniosynostoses have been measured and statistical tests performed to determine whether significant differences exist relative to the Abbott-Netherway data on normal ICV.

Abbreviations used in this paper: CT = computerized tomography; ICV = intracranial volume; SD = standard deviation.

TABLE 1

*Age characteristics (in months) for each group in study**

Craniosynostosis Diagnosis	Sex	No. of Measurements	Mean	Median	SD	Min	Max
metopic	F	7	4.4	2.6	4.2	1.8	13.6
	M	25	6.5	4.9	4.7	0.9	18.2
sagittal	F	11	3.3	2.9	1.1	2.0	5.7
	M	39	24.3	3.8	41.0	1.0	167.2
unicoronal	F	17	12.3	5.4	16.9	2.1	69.2
	M	11	7.1	3.6	7.8	1.7	25.8
bicoronal	F	3	2.7	2.6	0.2	2.6	2.9
unilambdoid	M	2	6.9	6.9	1.0	6.2	7.6
bilambdoid	F	2	46.7	46.7	44.8	15.0	78.3
bilambdoid & posterior sagittal	F	1	15.4	15.4	—	15.4	15.4
	M	2	1.7	1.7	0.4	1.4	1.9

* — = not applicable.

Clinical Material and Methods

Patient Selection

All patients with craniosynostosis presenting at The Australian Craniofacial Unit undergo CT scanning as a part of the management protocol. Patients with nonsyndromal craniosynostosis who had no surgical intervention prior to their CT scan were selected for ICV measurement. Table 1 gives the patient numbers and age distributions for each sex and diagnosis. The two measurements for patients with bilateral lambdoid synostosis were for the same patient.

Determination of ICV

For ICV measurement, the CT slices were processed one at a time to obtain the area of intersection of the region of interest with each slice. The in-house software package Persona was used to contour (that is, outline) the bone in each slice at the specified soft-tissue/bone threshold and to edit the contours.⁹ Triangulation of the contours was found useful for visualization and error detection and correction. A threshold of 150 Hounsfield units was selected for determination of the bone surface for the children in our study. The ICV was calculated as the sum of the cross-sectional areas that intersected the region of interest multiplied by the slice separation (referred to as the Cavalieri estimator). A bias correction term was applied to compensate for the effects of partial voluming, depending on slice thickness and separation. This method allowed us to use our archived CT scans, which had data acquisition resolutions (slice thicknesses) ranging from 1 to 5 mm.

Comparison With Normal Intracranial Volume

The Abbott-Netherway normal curves⁷ were used for comparisons. These were based on Ratowsky's¹¹ reparameterization of the three-parameter, asymptotic, regression-growth curve $y = a(1 - e^{-k(x-b)})$. The curves for each sex give the logarithm of the ICV as a function of the logarithm of the age from conception (y and x in the above equation, respectively). The use of the logarithm means that the coefficient of variation (SD/mean) was modeled as constant across the entire age range.

An ICV SD score for each patient was determined as the difference between the natural logarithm of the patient's

ICV and the sex-matched normal curve evaluated at the patient's age, divided by the SD. The SD score variable has an expected mean of zero and a SD of unity. Two-sample t -tests between the mean SD scores and F tests between variances were performed between each patient group and the sex-matched normal group.

Results

The SD scores for the ICVs of patients with nonsyndromal craniosynostosis are shown in Fig. 1, and their descriptive statistics are given in Table 2. The ICVs of all but two individuals were within the normal range of variation, and both of these were larger than normal.

Only the group of boys with metopic synostosis (Fig. 2) had significantly smaller ICVs than normal. Of the boys with metopic synostosis older than 7 months of age, only one was above the age- and sex-matched mean. Partitioning the male metopic data into age groups younger and older than 7 months of age revealed that the younger group had normal ICVs ($p = 0.339$, 17 patients), whereas the older group had statistically significant smaller ICVs ($p = 0.015$, eight patients).

The group of girls with metopic synostosis showed no indication of having smaller ICVs: only one patient was older than 7 months, and she had a positive SD score of 1.34.

Individually, patients with sagittal synostosis span the normal range of ICV (SD score range -2.2 to 2.1). Girls with sagittal synostosis (Fig. 3) have higher than normal ICVs ($p = 0.0002$), whereas those of boys are not significantly different from the normal values ($p = 0.231$). This result for sex is in contrast to that reported by Netherway, et al.,⁸ who used a smaller population.

The boys with unilateral coronal synostosis (Fig. 4) had larger than normal ICVs ($p = 0.001$). All but one had an

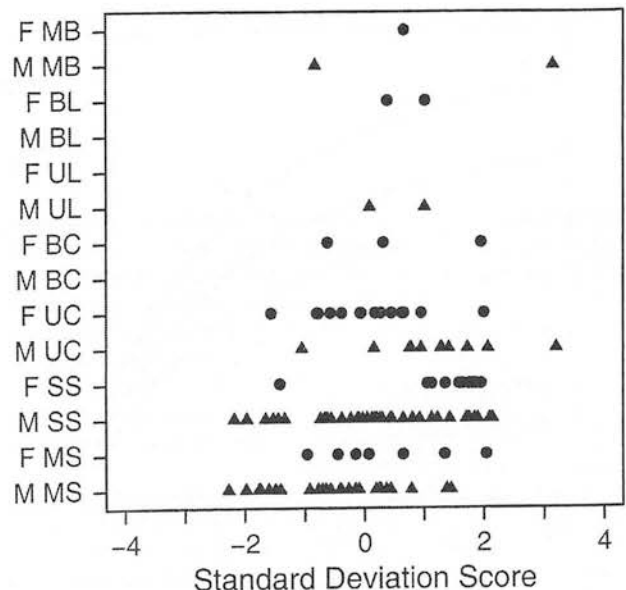


FIG. 1. Graph showing the SD scores for ICVs for male (M) and female (F) patients with nonsyndromal craniosynostosis: metopic (MS), sagittal (SS), unilateral coronal (UC), bilateral coronal (BC), unilateral lambdoid (UL), bilateral lambdoid (BL) and both posterior sagittal and bilateral lambdoid (MB).

TABLE 2
Standard deviation scores of ICVs for each group*

Craniosynostosis Diagnosis	Sex	No. of Measurements	Mean Score	Median	SE	Min	Max	p Value (t-test)	p Value (F test)
metopic	F	7	0.36	0.07	0.397	-0.96	2.04	0.361	0.735
	M	25	-0.48	-0.41	0.202	-2.27	1.45	0.040†	0.917
sagittal	F	11	1.25	1.58	0.285	-1.42	1.95	0.0002†	0.912
	M	39	0.26	0.20	0.192	-2.18	2.14	0.231	0.180
unicoronal	F	17	0.15	0.27	0.200	-1.56	2.00	0.552	0.388
	M	11	1.14	1.28	0.326	-1.05	3.20	0.001†	0.654
bicoronal	F	3	0.55	0.31	0.754	-0.62	1.95	0.359	0.377
unilambdoid	M	2	0.55	0.55	0.462	0.09	1.01	0.446	0.969
bilambdoid	F	2	0.70	0.70	0.318	0.38	1.02	0.329	0.691
bilambdoid & posterior sagittal	F	1	0.66	0.66	—	0.66	0.66	0.512	—
	M	2	1.16	1.16	1.988	-0.83	3.15	0.126	0.013†

* SE = standard error of the mean.
† Values are statistically significant ($p < 0.05$).

ICV larger than the mean normal ICV; the exception was the oldest in the sample at 26 months. The youngest had an exceptionally large ICV, with an SD score of 3.2. On review, there was no clinical reason to exclude this patient's data. When he was removed from the sample, the mean SD score was reduced from 1.14 to 0.94, the median score decreased from 1.28 to 1.11, and the probability value increased from 0.001 to 0.006, thus the high value has little impact on the finding of a larger than normal ICV.

Lambdoid synostosis is rare, and both sexes are not represented in our study. Our patients with unilateral and bilateral lambdoid synostosis had unremarkable ICVs, with SD scores in the range from 0.09 to 1.02. The patient with bilateral lambdoid synostosis whom we have measured at 15 months and at 6 years showed a slight relative increase in ICV, growing from SD score 0.38 to 1.02. She has had no surgical intervention.

Of the three patients with posterior sagittal and bilateral lambdoid synostosis (sometimes referred to as Mercedes-Benz syndrome⁷ because the sutural ridging pattern resembles the well-known automobile icon), two have intracranial volumes within the normal range, whereas one of the boys has an SD score of 3.15. The F test (see the last column of Table 2) indicated that the variance for the males in this group was greater than normal. The ICV variance for the other nonsyndromal groups did not significantly differ from the normal range. The ICVs of the patients with bilateral coronal synostosis were within the normal range.

Discussion

In the principal studies comparing the ICVs of patients with craniosynostosis and those of a reference of normal individuals, many authors questioned the validity of the ref-

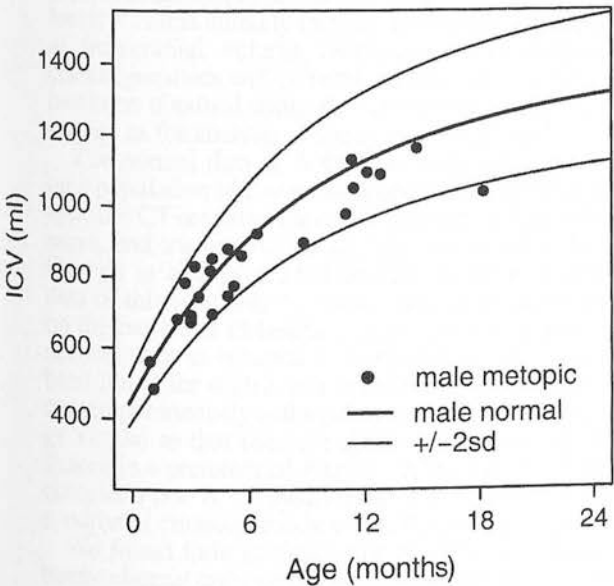


FIG. 2. Graph showing the ICVs for boys with metopic synostosis. For children younger than 7 months of age, the data are distributed within the normal range; for those older than 7 months of age, all but one point is below the mean for a normal population.

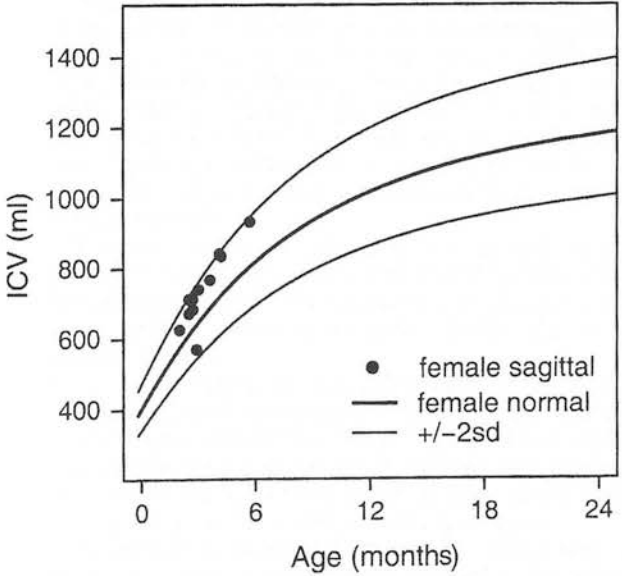


FIG. 3. Graph showing the ICVs for girls with sagittal synostosis. Although all patients had ICVs within the normal range, it is statistically significant that all but one had ICVs greater than the mean for a normal population.

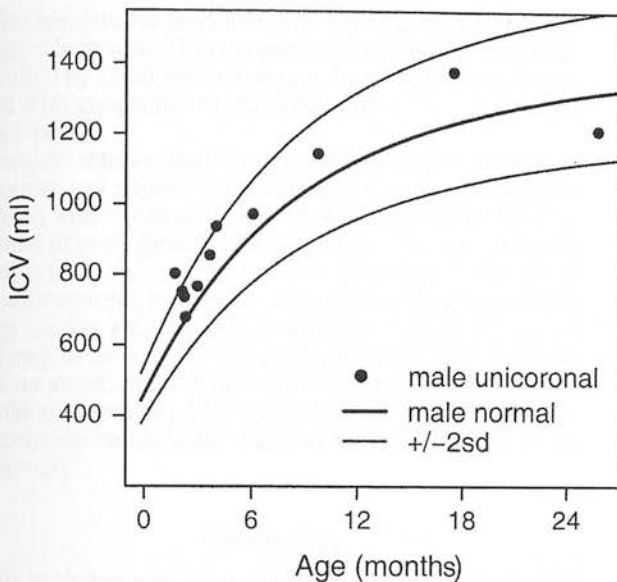


FIG. 4. Graph showing the ICVs for boys with unilateral coronal synostosis. It is statistically significant that all but one had ICVs greater than the mean for a normal population.

erence group. For example, Gault and colleagues⁴ make the following comment: "The significant difference between girls and boys found in this study was surprising and difficult to explain. It may be that the effect of craniosynostosis on skull growth is more pronounced in girls than boys. Alternatively, one could question the accuracy of the normal curves." Likewise, Posnick and colleagues¹⁰ stated "... we were surprised to find that our children's preoperative intracranial volumes were generally above age- and sex-matched norms." Marsh⁶ said of Posnick and colleagues' paper that it "... raises basic questions concerning the selection of comparative 'normal' data." He concluded as follows: "... it is unlikely that the controversy regarding actual intracranial volume measurements in patients with craniosynostosis will be resolved until a normative data set has been obtained using the same methodology and technology as for analysis of the synostosis group."

The normal data of Abbott and colleagues¹ were based on a population of European decent who had undergone diagnostic CT scanning for conditions such as headaches, seizures, and trauma that should not have affected their ICV. Caution is always needed because of the possibility that data of this type may be biased toward underdevelopment on the basis that ill health is more likely to impede development than to enhance it. Nevertheless, no evidence has been found for such a bias in their data. The CT data were contemporaneously collected using protocols and technology similar to that used for clinical assessment of patients treated in a craniofacial surgery department. Here we have compared our ICVs measurements for patients with nonsyndromal craniosynostoses with this normal population.

We found little evidence that the ICV of patients with nonsyndromal craniosynostosis are smaller than normal except for boys with metopic synostosis. Only two individuals, a boy with sagittal synostosis and a boy with metopic synostosis had SD scores less than -2. The mean for male patients with metopic synostosis tended to be less than nor-

mal ($p = 0.04$). This finding for metopic synostosis was reported by Anderson, et al.,² using the same data as reported here. Further inspection of the data revealed that it was the patients older than 7 months of age who contributed to this low ICV finding. If very young male patients with metopic synostosis indeed have normal ICVs and the older group have reduced ICVs (on average), then these findings could have implications for management of these patients. If a patient group were found to have smaller ICVs than a normal reference group, it may be that that group is simply underdeveloped in regard to the reference group rather than as a direct result of the pathological findings. Because these children as a group have normal ICVs before the age of 7 months and then have smaller ICVs on average after 7 months, an association between the pathological entities and ICV is indicated; however, the impact on brain development remains uncertain. This impact may be clarified by further cases and by longitudinal follow up.

For patients with metopic synostosis, Gault, et al.,⁴ reported reduced—although not statistically significant—ICVs relative to the Lichtenberg normal group⁵ for five boys and three girls, and Posnick, et al.,¹⁰ reported larger ICVs than the Lichtenberg group for seven boys and three girls, whereas Marsh⁶ reported ICVs for four patients within one SD of the Lichtenberg group. Our findings are more in keeping with those of Gault, et al., and of Marsh.

All patient groups with nonsyndromal craniosynostosis other than boys with metopic synostosis had positive mean SD scores, meaning that, on average, patients in our sample had larger than normal ICVs; however, this was statistically significant only for girls with sagittal synostosis and boys with unilateral coronal synostosis. Both of these groups had a sample size of 11. It is possible that a larger sample size may show that these groups do not differ from normal ones, but the evidence from this study is that the average ICV for patients in these groups is larger than normal.

For patients with sagittal synostosis, Gault, et al.,⁴ reported normal ICVs relative to the Lichtenberg group⁵ for 30 boys and 16 girls, and Posnick, et al.,¹⁰ reported larger ICVs than the Lichtenberg group for six boys and two girls, whereas Marsh⁶ reported ICVs for five patients within one SD of the Lichtenberg group. Our findings for our sagittal synostosis groups were that their ICVs are in the normal range but have a bias toward being larger than normal for girls.

It is notable that the age range for our study group of girls with sagittal synostosis is up to 5.7 months, whereas the unoperated boys in our database extend in age up to 14 years, perhaps indicating a previous preference for intervention for girls.

As stated earlier, we found that the ICV for boys with unilateral coronal synostosis tended to be larger than found in the Abbott-Netherway normal group. Gault and colleagues⁴ did not report separately for boys and girls but indicated that the ICVs were less than those of the Lichtenberg group for patients with coronal synostosis.

Differences between our findings and others (using Lichtenberg's normal curves) can be accounted for partly by consideration of the age- and sex-dependent differences between the Lichtenberg and the Abbott-Netherway normal curves, and partly by sample differences from the relatively small sample sizes in studies on this topic. Lichtenberg

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pooled his data for boys and girls younger than 12 months of age, which would have contributed to the sex differences identified by Gault and colleagues. Much of the data for patients with unoperated craniosynostosis is for ages younger than 1 year.

Surgical intervention for craniosynostosis is undertaken to correct and prevent further distortion of the craniofacial skeleton and because of the attendant potential for constriction of brain growth. For all groups with nonsyndromal craniosynostosis, other than boys older than 7 months of age with metopic synostosis, there is no indication of ICVs being smaller than normal; in addition, two groups have a tendency to have ICVs that are larger than normal. Therefore, intervention for these nonsyndromal craniosynostoses should appropriately be focused less on ICV and more on normalizing craniofacial shape and promoting normal development.

Conclusions

No evidence was found that the ICVs of patients with nonsyndromal craniosynostosis are smaller on average than those of normal patients, except for boys with metopic synostosis. Both the groups of girls with sagittal synostosis and the boys with unilateral coronal synostosis had larger than normal ICVs. That the boys with metopic synostosis have normal ICVs and the older group have reduced ICVs (on average) may have implications for the timing of surgical intervention for these patients, although the small sample size for the older group suggests that the addition of further cases would be prudent to validate the tentative association. The indications are that surgical intervention should focus less on ICV and more on normalizing craniofacial shape and promoting normal development.

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Oral features in Apert syndrome: a histological investigation

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Structured Abstract

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Objectives – The number of publications on the oral features in Apert syndrome is limited. The present study investigated dental tissues in Apert syndrome histologically, to determine the nature and extent of anomalies, to provide some insight into the nature of the condition, and to explain how observed anomalies may affect the dental management of individuals with Apert syndrome.

Setting and Sample Population – Extracted primary and secondary teeth were collected from patients with Apert who had attended the Australian Craniofacial Unit, Adelaide, South Australia. The total study sample comprised 13 individuals, aged from 14 to 21, with nine men and four women.

Material and Methods – A total of 40 teeth were available for histological examination (the number belonging to each individual varied from 2 to 5 per patient). The teeth were sectioned longitudinally, and one-half of each tooth underwent decalcification. Sections were stained with H&E for routine histological examination. Ground sections were prepared from undecalcified tooth halves.

Results – Histological assessment of the dental hard tissues revealed an intact enamel and dentinal structure but some irregularities were noted in the region of the dentino-enamel junction (DEJ), which could affect caries progression and also make dental management more difficult.

Conclusion – This study identified histological anomalies of the DEJ of Apert syndrome teeth. An improved appreciation of the nature and extent of dental anomalies in Apert syndrome should assist clinicians when undertaking management of affected individuals.

Key words: Acrocephalosyndactylia; craniosynostoses; dentino-enamel junction; dentistry; tooth

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Introduction

Apert syndrome is an autosomal dominant disorder that was first described by Wheaton in 1894 (1). Subsequently, it was given its eponymous name after being described by Apert in 1906 (2). It is a form of craniosynostosis, a condition in which there is pre-mature closure of certain calvarial sutures. The coronal suture is specifically affected in Apert syndrome, leading to characteristic head shapes secondary to restriction of growth perpendicular to the suture and compensatory growth in

the remaining sutures. This produces a wide midline defect affecting the area of the metopic and sagittal sutures. Apert syndrome is an example of a syndromic craniosynostosis, where an underlying genetic mutation has been identified, and multiple sutures are affected along with other manifestations.

The prevalence of Apert syndrome has been reported as 1:200 000, constituting a small proportion of all cases of craniosynostosis, and it occurs equally in men and women in population-based studies, but clinical studies have revealed more affected women (3). Despite its rarity, Apert syndrome has been studied by more authors than any of the other three craniosynostosis syndromes. The triad of features that is associated with Apert syndrome includes craniosynostosis, turribrachycephaly and midface hypoplasia (4). Gorlin (5) described the head as being broad, with the metopic and sagittal sutures widely patent during infancy, resulting in turribrachycephaly (towering skull). The characteristic craniofacial features of Apert syndrome are shown in Fig. 1. Common findings include maxillary hypoplasia, which results in shallow orbits and proptosis, hypertelorism and down-slanting palpebral fissures, along with syndactyly of the hands and feet (4). There have been reports of affected individuals having anomalies of the viscera, elbows and shoulders, skeleton and central nervous system, and this often results in impaired mental function, emphasizing the need for ongoing care by a wide range of medical and surgical specialists.

The clinical and genetic factors associated with Apert syndrome have been studied extensively. The majority of studies have focused on the frequency and resulting effects of the two major types of mutations affecting the FGFR2 gene. Park et al. (6) summarized the genetic and environmental interactions in Apert syndrome. The mutations in the resulting FGFR2 gene alter the three-dimensional shape of the receptor and affect its role in growth and development, resulting in pre-mature fusion of bones in the skull, hands and feet of the patient with Apert. During craniofacial development, FGF signalling is involved not only in craniofacial bone formation, but also in the formation of the palate, salivary glands, teeth, craniofacial muscles and tongue muscles (7). It has been well documented that FGFR2 also plays an important role in tooth development. For example, it has been shown that in mice with FGFR2 mutations, tooth development fails to develop beyond the bud stage, with defects in the salivary glands and palate also being recorded (7). Therefore, it seems feasible that FGFR signalling mutations influencing the craniofacial features in Apert syndrome might also influence the dental tissues, leading to alterations in size and shape of the teeth. Furthermore, FGF signalling plays an important role in the epithelial-mesenchymal tissue interactions that occur normally between the inner enamel epithelium and the dental papilla during odontogenesis, and altered expression of this growth factor at critical stages of development in Apert syndrome could lead to a range of effects on the dentition.

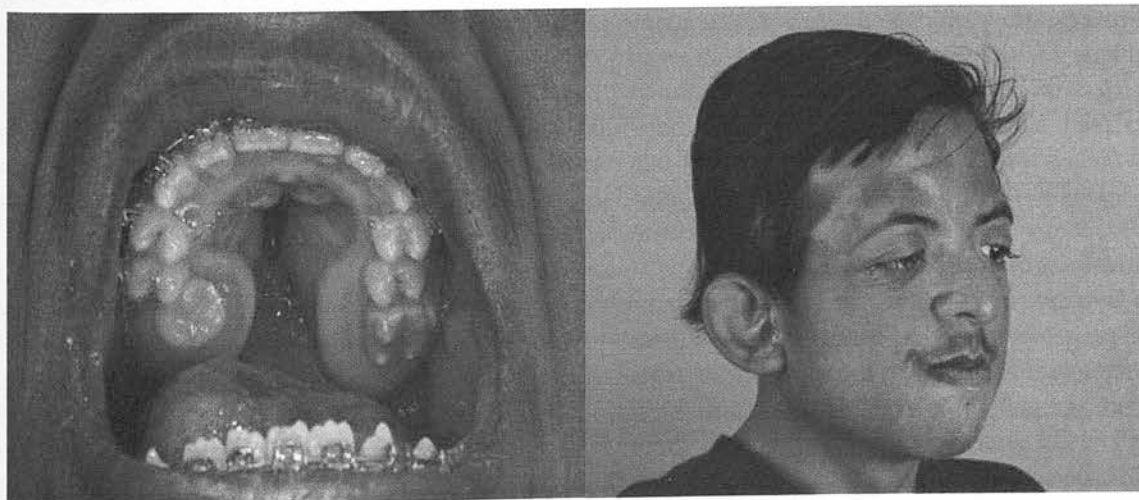


Fig. 1. Intra- and extra-oral photographs of a male with Apert syndrome.

Craniofacial features of Apert syndrome will always be an area of focus in the literature, as developments made can help to improve function, appearance and ultimately the long-term survival of the patient with Apert. With advances in medical imaging, such as 3D-CT scanning and in-utero imaging, greater details of the craniofacial skeleton can be obtained. The oral and dental manifestations of the syndrome have been described in only a few studies that have focused on macroscopic features of the syndrome (8, 9). Various studies have recently identified similar intraoral characteristics, including a crossbow or trapezoid-like mouth shape with often protruding lips, a hypoplastic maxilla that appears retruded and thus a Class III malocclusion with an edge-to-edge incisor relationship and anterior open bite (10–12). Delayed tooth eruption times of almost a year have also been reported. Specific dental anomalies were reported by Dalben et al. (12), including enamel projections, long canine cusps, supernumerary teeth, dental fusion, enamel opacity, enamel hypoplasia, tooth agenesis, ectopic tooth positioning and tooth impaction.

Common trends have emerged from previous studies of Apert syndrome, with anomalies in growth of the calvaria and facial skeleton being an important area for investigation. Considerable numbers of patients have been studied across a range of ethnicities, involving children of different ages. This provides a valuable clinical spectrum of the variation seen in the craniofacial region and the common skeletal defects of Apert syndrome. However, Apert syndrome is a rare disorder, and consequently sample sizes reported in previous individual studies have been small. Therefore, limited data are available for quantitative comparisons with findings often based on single case presentations that authors have attempted to associate with the entire spectrum of Apert syndrome. Generalization of typical features associated with Apert syndrome can be made to some extent, but each individual has a unique presentation that needs to be taken into account when management strategies are developed.

Apart from a study by Solomon et al. (13) on mucopolysaccharides in the palatal mucosa of patients with Apert, information on the histological appearance of the oral tissues in Apert syndrome is scarce. This study aims to provide more definitive insights into the histological structure of the dental tissues in Apert

syndrome. The knowledge gained from this study should alert dental practitioners to the challenges that may arise when managing oral problems in patients with Apert syndrome.

Materials and methods

Sample population

This study involved 13 patients with Apert syndrome for whom records are kept in the Australian Craniofacial Unit (ACFU) at the Women's and Children's Hospital, Adelaide, South Australia. Craniosynostosis syndromes, including Apert syndrome, are a major area of focus in both surgical and multidisciplinary management by the ACFU, and thus a relatively high number of patients have been treated with this condition despite its rare nature. Ethics approval was obtained from the Ethics Committee at the WCH (WCH200A).

Extracted teeth for histological study

Teeth of patients diagnosed with Apert syndrome that had been extracted previously following ACFU surgical procedures in association with each patients particular management protocol formed the study sample for histological analysis. Forty teeth stored in formalin were evaluated for study, including both primary and secondary dentitions.

Each tooth was bisected longitudinally. One-half of each tooth underwent decalcification and was processed for routine histological examination. Five micron sections were stained with haematoxylin and eosin (H&E). Ground sections were prepared from the remaining undecalcified tooth halves enabling examination of both the enamel and dentine. All specimens were examined using an Olympus BH2 microscope and photographed using an Olympus Altra 20 digital camera (Olympus Australia, Mt. Waverley, Victoria, Australia).

The images provided in this article represent only a small proportion of the samples prepared and examined. It should be noted that, to avoid sampling bias, all areas of tooth structure visible in histological analysis were assessed and noted with features being consistently described. Images that were chosen for inclusion as figures provided the best representations of the features under study.

Results

Examination of the dental hard tissues of extracted teeth from Apert syndrome individuals revealed no gross anomalies in enamel or dentinal structure. Figures 2 and 3 show the relatively normal dentinal appearance of an patient with Apert syndrome. The enamel also appears normal despite the difficulties in imaging because of optical light interference.

When the dentine and enamel were examined at higher magnification, the DEJ appeared to show some inconsistencies within its structure. The literature is clear in describing the DEJ as normally being scalloped in appearance in ground sections and showing marked scalloping in demineralized sections (14). However, all of the specimens examined failed to show this feature. Histological sections showed either a flat DEJ or a wavy, inconsistent junction that did not conform with the structure usually described (Fig. 4).

Other structures seen in the enamel included enamel tufts and lamellae, which are developmental features relating to the formation of the enamel and dentine whose structure is influenced by the DEJ formation. Four specimens, or 10% of the teeth cut in a mesio-distal plane showed irregularities including small dark projections from the dentine into the enamel. Some areas appeared with no projections and some tufts/lamellae were present (Fig. 5). There was also evidence of a broken DEJ in a few Apert syndrome

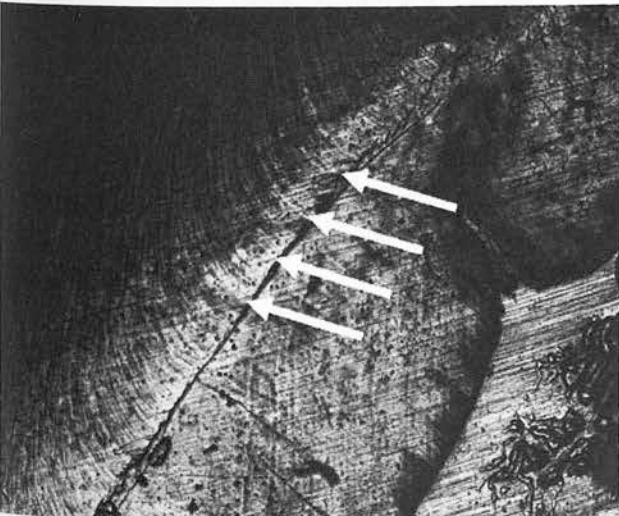


Fig. 2. Ground section of an Apert syndrome tooth (×10 magnification). Note the characteristic curvature of the dentinal tubules approaching the dentino-enamel junction (see arrows).

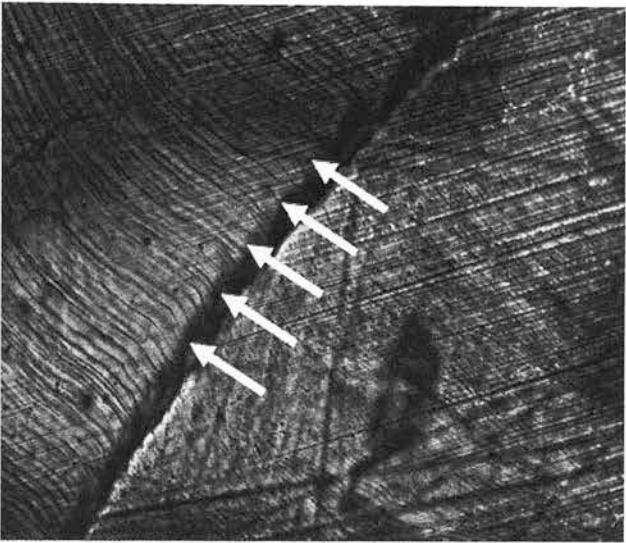


Fig. 3. Ground section of an Apert syndrome tooth (×40 magnification). Note the defined tubular structure of the dentine (see arrows).

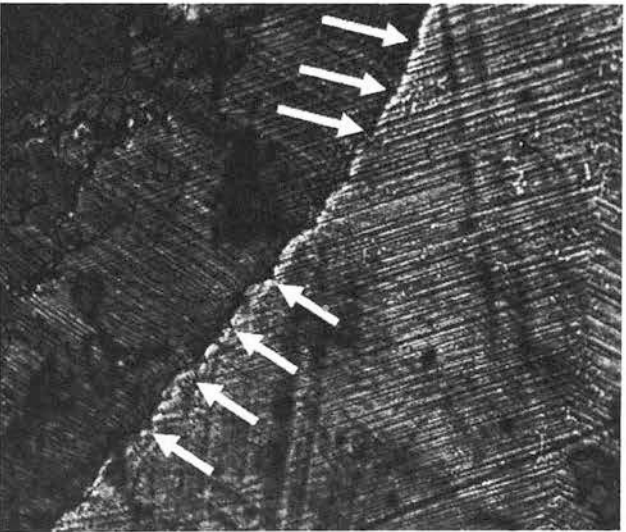


Fig. 4. ×10 microscopic view of a ground section showing inconsistent structure at the dentino-enamel junction. Scalloping is non-existent towards the top of the image (top arrows) and is marked towards the bottom of the image (bottom arrows).

specimens, with the large gaps appearing to be artifacts that had occurred during preparation (Fig. 6).

H&E stained sections revealed a relatively intact dentinal structure. The tubular structure appeared to be normal and did not show any obvious irregularities when examined histologically. However, the DEJ again revealed variation. It showed a profile that differed from descriptions in the literature. Scalloping was rarely seen in the Apert specimens and, if present,

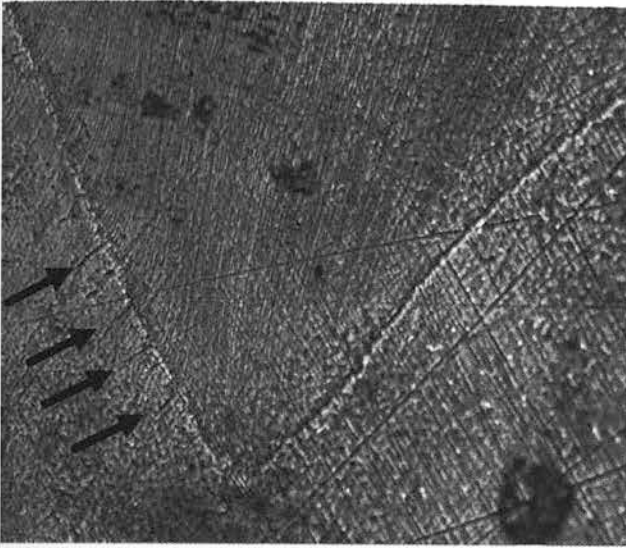


Fig. 5. $\times 20$ microscopic view of a ground section showing some evidence of projections or lamellae into the enamel from the dentino-enamel junction. This is more marked on the left of the image (see arrows).

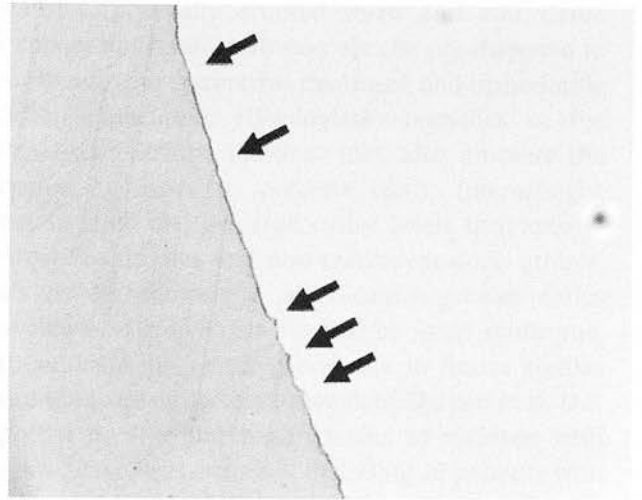


Fig. 7. $\times 10$ microscopic view of the dentine (darker area) and dentino-enamel junction (DEJ) (see arrows), which shows a flattened DEJ with some irregular scalloping. The lighter area identifies the demineralized enamel space.

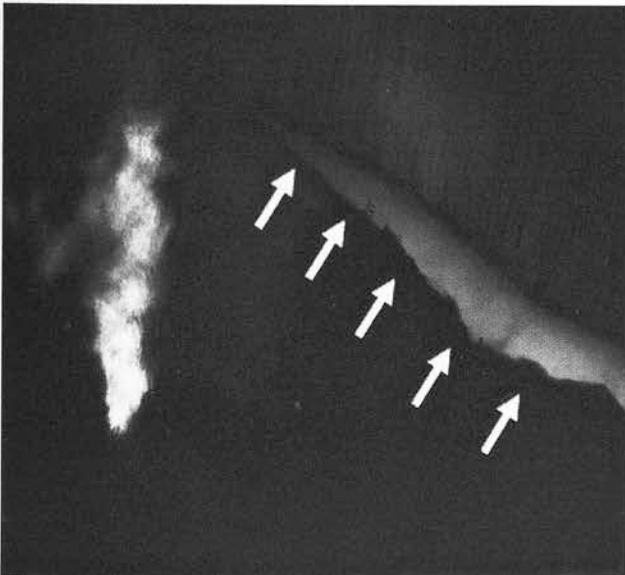


Fig. 6. $\times 10$ microscopic view of a ground section showing marked spacing between the enamel and dentine structures at the dentino-enamel junction, identifying a defect in section preparation (see arrows).

showed an inconsistent or irregular pattern (Figs 7 and 8).

Discussion

Despite considerable documentation on the general features of Apert syndrome, there is little published

literature on the oral effects of the syndrome. This study has provided some new insights into the histological features of the dental tissues in Apert syndrome. Given the rarity of the condition, a relatively large sample of Apert teeth was available for study.

An early description of the DEJ was given by Tomes (15), who implied that all enamel is festooned (showing a looped or curved appearance) towards the dentinal surface. Few studies have questioned this concept, although Rywkind (16) noted that in some teeth the scalloping is irregular in size and distribution and sometimes absent. Gustafson (17) confirmed the basic arcade-shaped appearance of the junction, but agreed that the development of the scallops varied from tooth to tooth, and suggested that the pattern may be characteristic of the individual. The authors noted that the arcades were more pronounced in fluorosed teeth. Falin (18) described the DEJ in Bronze Age teeth as being flat or slightly festooned in pre-molars and molars, and scalloped in canines and incisors. No comparable study appears to have been carried out in teeth of modern origin. Scott and Symons (19) commented upon the variation in size of the dome-shaped scallops, which are usually most marked in the cuspal region, but are occasionally absent. The location of the most marked scalloping, as described by Schour (20), was in the gingival third of teeth. Whittaker et al. (21) found tufts and lamellae commonly within specimens of normal tooth structure but, in our

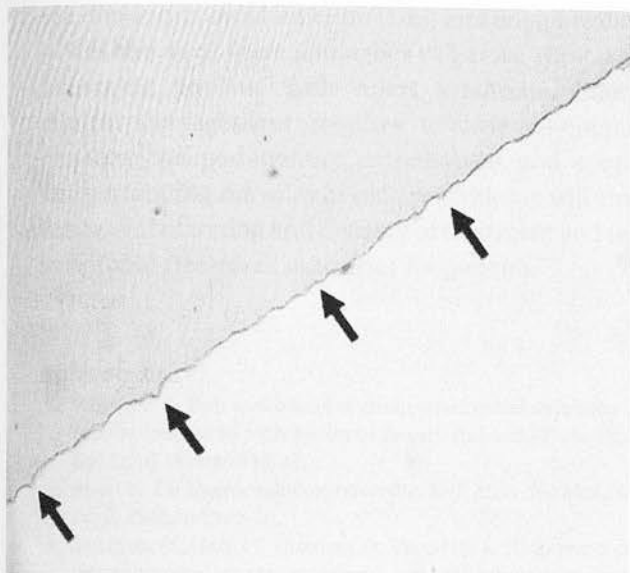


Fig. 8. Molar cervical region of an Apert syndrome tooth that has been H&E prepared. Note the irregular scalloping along the entire length of the dentino-enamel junction. The arrows show some of the more pronounced irregularities.

study, tufts and/or lamellae were rarely visible (only appearing in 10% of samples). This may suggest anomalies associated with DEJ development. In our study, scalloping was absent or irregular in all areas of the crown and seemed consistently absent in the cuspal regions, where it has previously been described as being profound. The preparation of the samples caused some trauma to specimens, especially during the hand polishing stages, and this may have contributed to the artifacts seen.

Given that FGF signalling (particularly FGFR2) plays an important role in the formation of the teeth, and formation of the enamel and dentine is dependent on reciprocal epithelial and mesenchymal interactions, anomalies in FGFR signalling in Apert syndrome may affect dental development, leading to macroscopic and histological anomalies (22, 23).

So what do these findings mean for patients with Apert syndrome? They suggest that changes should be considered to optimize the management of children with Apert syndrome, both dentally and orthodontically. Anomalies in the teeth of patients may predispose them to difficulties in tooth eruption, in maintaining good oral hygiene and in ensuring correct bonding of orthodontic brackets and placement of orthodontic wires. Hohoff et al. (10) reported on the difficulties associated with orthodontic treatment

because of partially erupted teeth and soft tissue anomalies but Apert teeth may also be pre-disposed to poor bonding in restorative treatment and orthodontic bracket application. Histological anomalies of the enamel and dentine interface may also promote the advance of caries in patients teeth. Interestingly, Mustafa et al. (24) assessed caries levels in craniosynostosis syndromes and non-craniosynostosis groups, with results showing a significantly greater caries prevalence in individuals affected by Apert syndrome. Further focus on caries prevalence in future studies should provide more conclusive data. Dalben et al. (12) reported on the oral health status of children with syndromic craniosynostosis, including 10 patients with Apert syndrome, and showed a predominance of caries (measured by DMFT (Decayed, missing, filled teeth) scores) in these patients, concluding that there is an increased need for follow-up programmes by their dental practitioners. Anomalies in the DEJ interface may also affect the ability of materials to bond to the enamel and dentine, thus increasing the likelihood of de-bonding of materials while also increasing the risk of secondary caries. Further studies of the caries experience of patients with Apert would be valuable in ensuring that this risk is reduced and that appropriate management plans are implemented.

Overall, the findings of the present investigation indicate that there are anomalies in the oral hard tissues in patients with Apert syndrome. Further studies comparing the histological characteristics of teeth obtained from individuals with Apert syndrome with teeth obtained from unaffected individuals would further strengthen the findings. Although the study sample was limited in size because of the rarity of the syndrome, results showed that the dental tissues vary from normal histologically, and these variations have implications for the management of individuals with Apert syndrome.

Clinical relevance

Children with Apert syndrome have obvious dysmorphic facial growth, particularly affecting the midface. The need for corrective surgery, therefore, is inevitable. The orthodontist faces challenges because of anomalies in the morphology of the teeth, crowding and delayed eruption. This study identified histological anomalies in

the dentino-enamel junction (DEJ) and an appreciation of the effects of these anomalies will assist clinicians in managing patients with Apert syndrome. Effective clinical management requires a close co-operation between the pedodontist, orthodontist and surgeon. Understanding the roles of each practitioner will ensure successful planning and delivery of treatment and result in optimal treatment outcomes for patients.

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ORIGINAL ARTICLE

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Identification of genes differentially expressed by prematurely fused human sutures using a novel *in vivo*–*in vitro* approach

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Abstract Craniosynostosis is the premature fusion of calvarial sutures. It results from abnormal differentiation or proliferation of cells within the osteogenic fronts of growing calvarial bones. To date, research has focused on animal models and *in vitro* organ and tissue culture to determine the molecular mechanisms con-

trolling calvarial suture morphogenesis. Here, we test a new, *in vivo*–*in vitro* approach based on the hypothesis that calvarial suture cells passaged in minimal medium exhibit a stable gene expression profile similar to undifferentiated osteoblastic cells that can provide a benchmark for comparison with *in vivo* expression of differentiated tissue. We show that tissue-specific expression is lost after the first passage and, using cDNA microarrays, compare expression between fused suture tissue from craniosynostosis patients and *in vitro* de-differentiated explant cells. A large number of differentially expressed genes were identified, including novel genes *WIF1*, *LEF1*, *SATB2*, *RARRES1*, *DEFA1*, *DMP1*, *PTPRZ1*, and *PTPRC*, as well as those commonly associated with human suture morphogenesis, e.g., *FGF2*, *MSX2*, and *BMP2*. Two differentially expressed genes, *WIF1* and *FGF2*, were further examined in an *in vivo*–*in vitro* comparison between unfused and prematurely fused tissue. The same pattern of differential expression was observed in each case, further validating the ability of our *in vivo*–*in vitro* approach to identify genes involved in *in vivo* human calvarial tissue differentiation.

Key words craniosynostosis · microarray · differentiation · osteoblast · de-differentiate.

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Introduction

Craniosynostosis, the premature fusion of calvarial sutures, is the second most common craniofacial developmental abnormality, occurring in one in 2,500 live births, and results in cranial vault and facial malformation (Wilkie, 2000). Premature suture fusion results from abnormal differentiation and proliferation of cells within the growing osteogenic fronts of calvarial

bones, an area populated by mesenchyme, osteoprogenitor cells, preosteoblasts, and osteoblasts. The study of premature fusion thus provides a model to study the molecular process of osteogenesis. To date, 11 genes have been identified with mutations causing craniosynostosis including members of the fibroblast growth factor (FGF), transforming growth factor β (TGF β), and Eph/Ephrin families (for a review, see Rice, 2005). The number of genes and signaling pathways so far identified emphasize the complexity of the molecular regulation of suture development.

A number of approaches have been used to study the molecular pathogenesis of craniosynostosis. These include the analysis of transgenic animals expressing orthologous human craniosynostosis mutations, *in vitro* manipulation of organ culture or cell lines generated from animal calvaria, or *in vitro* culturing of human suture tissue obtained from patients with craniosynostosis (for example, Bourgeois et al., 1998; Liu et al., 1999; Opperman et al., 2002a; Guenou et al., 2005; Mansukhani et al., 2005; Ratisoontorn et al., 2005; Sahar et al., 2005). The findings of these studies relate either to the *in vivo* development of non-primate animals, or they have been conducted in an *in vitro* environment using various media that promote either cellular proliferation or artificial induction of a phenotype that resembles osteoblast differentiation. Thus, it is uncertain how the experimental observations from these studies relate to the *in vivo* processes of premature suture fusion in humans.

There are a number of approaches that are more appropriate when investigating the human situation. The approach with the least ambiguity in interpretation is an *in vivo*–*in vivo* comparison of fused, fusing, and unfused regions of the same suture from the same craniosynostosis patient. Such *in vivo* comparisons have been successful for the murine situation (Most et al., 1998; Spector et al., 2000; Song et al., 2004; Sahar et al., 2005); however, such an analysis in humans is associated with significant ethical and technical challenges. Moreover, as these are complex tissues the degree of differential gene expression will be less than that when comparing, for example, undifferentiated and differentiated cells, as the tissue complexes are made up of cells of varying states of differentiation. The most commonly utilized approach for human studies is an *in vitro*–*in vitro* comparison between cells cultured from fused and unfused sutures, which allows not only an analysis of differential gene expression but also functional analysis of the differentiative and proliferative capacity of the different cell populations (de Pollack et al., 1996, 1997). However, the artificial nature of the *in vitro* environment creates difficulties in translating the results of an *in vitro*–*in vitro* comparison with the *in vivo* state. A number of studies have therefore subsequently utilized histological analyses of human suture tissues to confirm *in vitro* observations (Lomri et al., 1998; Lemonnier et al., 2000, 2001).

However, an interesting and potentially advantageous observation from *in vitro* manipulations is that osteoblasts have the potential to de-differentiate under the right conditions (Owen et al., 1990; Nefussi et al., 1997; Lee et al., 1999; Song and Tuan, 2004). In particular, the removal of the extracellular matrix (ECM) from mature mineralized rat calvarial-derived osteoblasts *in vitro* through trypsinization results in the reinitiation of the developmental sequence of proliferation, osteoblast differentiation, ECM maturation and mineralization mediated through culturing in osteogenic media (Owen et al., 1990). Osteoblast cells expressing late-stage differentiation markers *osteocalcin* (*OC*) and *osteopontin* (*OP*) when grown in mineralization media and then trypsinized and grown in minimal medium undergo a rapid down-regulation of *OC* and *OP* expression, the expression of these markers being restored only after addition of osteogenic media and the reformation of a mature mineralized matrix. This demonstrates that mature osteoblasts can be induced to re-enter the developmental cascade.

Consistently, it has been shown that culturing of human calvarial suture cells isolated by collagenase digestion produces a population of cells with characteristics of preosteoblastic cells (de Pollack et al., 1997). The growth of such cells under basal conditions can be used to study progenitor proliferation, while growth under mineralization media is used to study the processes and temporal gene expression changes during osteoblast differentiation (de Pollack et al., 1996, 1997; Lomri et al., 1998; Yousfi et al., 2001; Ratisoontorn et al., 2005). Thus, to identify the genes involved in osteogenesis, it would be informative to compare calvarial cells grown under basal conditions with those at different stages of *in vitro* differentiation. Such a comparison has been carried out using gene expression microarrays to identify the genes involved in BMP2-induced osteoblastic differentiation of human mesenchymal stem cells (Vaes et al., 2005). However, it still remains unclear how fully reflective *in vitro* differentiated osteoblasts are of *in vivo* differentiated osteoblasts.

While both *in vitro* and *in vivo* analyses of human sutures have been used to investigate the pathophysiology of craniosynostosis, a comparative analysis of these two experimental systems has not been conducted for the purpose of gene identification. Here, we describe a novel application of human calvarial cell culture utilizing the observation that passage of human calvarial suture cells results in the production of a cell population characteristic of preosteoblastic cells. Microarrays were used to compare *in vivo* gene expression profiles of prematurely fused suture tissues with profiles of cells derived from the same tissue grown under non-differentiating conditions. Thus, we are able to compare the expression of fully differentiated tissue with that of cell populations exhibiting a gene expression profile typical of undifferentiated osteogenic cells.

Therefore, genes that are required to drive osteoblast differentiation and mineralization in the suture tissue will be expressed in the tissue samples but not by their corresponding matched, de-differentiated cell populations. Similarly, those genes found to be down-regulated in the tissue, compared with culture, are likely to be involved in inhibition of differentiation or inducing osteoprogenitor proliferation, the processes that precede bone formation and suture fusion. Finally, we validated our novel *in vivo-in vitro* approach by analyzing the expression of two identified genes in an *in vivo-in vitro* comparison between unfused and prematurely fused suture tissues. We show that the same pattern of differential expression is seen for the *in vivo-in vitro* and *in vivo-in vitro* analyses and that a greater fold change is observed for our novel *in vivo-in vitro* comparison, facilitating easier identification of differentially expressed genes.

Materials and methods

Suture samples

Calvarial suture samples were obtained from three patients undergoing transcranial surgery for syndromic or non-syndromic craniosynostosis. To minimize any age-related changes in osteoblast differentiation and bone formation (de Pollack et al., 1997) and to eliminate any sex-related effects on the development of craniosynostosis (Lajeunie et al., 1995), sutures were obtained from males aged 6–7 months. Patients were previously genotyped for all known *FGFR1-3* and *TWIST1* craniosynostosis-causing mutations (Table 1) (Anderson et al., 2007). Consent was provided by all guardians in line with the guidelines received from the Research Ethics Committee of the Children, Youth and Women's Health Service, Adelaide, South Australia. Suture tissue was taken from prematurely fused sutures, and one unfused suture from one patient (Table 1). Samples were sectioned into two parts on removal. Specimens used for cell culture were placed in Ringers solution for up to 4 h until processed. Specimens used for microarray analysis were stored in RNAlater (Ambion, Austin, TX). The suture complex (suture mesenchyme plus 3 mm bone on either side for unfused sutures, or fused bony ridge plus 3 mm bone on either side for fused sutures) was dissected from all specimens and the overlying pericranium was removed.

Suture cell culture

Human calvarial suture cells were obtained by collagenase digestion and explant culture following the method described by de Pollack

et al. (1996). Briefly, dissected suture samples were minced into 1 mm fragments and incubated in 0.25% collagenase for 2 h at 37°C. Samples were centrifuged and the supernatant was removed. Following three washes in PBS, samples were plated at 5 bone fragments per well, in 12-well plates, and cultured in high-glucose Dulbecco's modified essential medium (DMEM, Invitrogen Life Technologies, Gaithersburg, MD) supplemented with L-glutamine (584 mg/l), 10% fetal calf serum, and 1% antibiotics (penicillin 100 IU/ml, streptomycin 100 µg/ml) in a humidified atmosphere of 5% CO₂ maintained at 37°C. Upon confluence, cells were plated in T25 flasks and labeled P1. Medium was changed every 2 days. Cells were passaged to P4 and grown to 90% confluence before RNA extraction. For one fused sample (#58), RNA was also extracted before each passage.

Suture cell differentiation

To test the osteogenic capacity of cultured suture cells, at confluence (day 6) minimal medium was replaced with either minimal medium or minimal medium supplemented with 0.05 mM ascorbic acid, 10 mM β-glycerophosphate, and 100 nM dexamethasone. Media were changed every 3 days. Matrix formation and mineralization were measured at day 22, 16 days after the addition of supplemented media. Cells were washed in distilled H₂O, followed by staining for mineral formation using the von Kossa method and then for collagenous ECM formation using the van Gieson method, followed by fixation in 70% ethanol. RNA was extracted from triplicate unstained wells for each medium.

Total RNA isolation

Total RNA was isolated from P4 cells following trypsinization and washing with PBS. Cell pellets were resuspended in TRI Reagent (Molecular Research Center, Cincinnati, OH) and RNA was isolated following the manufacturer's protocol. Tissue samples stored in RNAlater were cut into 30–40 mg pieces. Individual pieces were wrapped in a heavy-duty aluminum foil, snap-frozen, crushed, and homogenized in 2 ml TRI Reagent using a Mini-Bead-Beater-8 (BioSpec Products, Bartlesville, OK). RNA was isolated from the supernatant following Naderi et al. (2004). Briefly, the separated aqueous phase was extracted twice with chloroform and precipitated with 1 volume isopropanol, 0.1 volume 7.5 M ammonium acetate, and 5 µg/ml linear polyacrylamide (Ambion) at –20°C overnight. Pelleted RNA was washed twice with 70% ethanol and resuspended in The RNA Storage Solution (Ambion). P4 cell RNA used for microarrays was treated with 10 Units DNase I (Qiagen, Clifton Hill, Australia) at a concentration of 5 µg RNA/100 µl. RNA extracts from the same tissues were combined and 10 µg of each combined tissue-RNA and treated-cell RNA was purified and concentrated with phenol:chloroform:isoamyl alcohol (25:24:1) extraction. Total RNA quality was determined by running a non-denaturing 1.5% agarose Tris-borate EDTA (TBE) buffered gel and analyzing the integrity of the 28S and 18S ribosomal bands in

Table 1 Suture samples used for analysis of tissue and explant cell expression by microarray and QRT-PCR

Patient	Phenotype	Sex	Age (months)	Identified mutation	Fused	Unfused
#42	Apert syndrome	M	7	FGFR2 Ser252Trp	Coronal	
#50	Sagittal synostosis	M	6		Sagittal	
#58	Sagittal synostosis	M	7		Sagittal ¹	Sagittal ¹

¹Samples obtained from these sutures used only for QRT-PCR.

QRT-PCR, real-time quantitative reverse-transcriptase polymerase chain reaction.

Table 2 Primers used for QRT-PCR

Primer set	Forward (5'-3')	Reverse (5'-3')	Amplicon (bp)
COL1A1	CGAAGACATCCCACCAATCAC	TTGTCGCAGACGCAGATCC	99
ALP	CGTGGCTAAGAATGTCATCATGTT	TGGTGGAGCTGACCCTTGA	90
BSP	TTTCTGCTACAACACTGGGCTATG	TTGAGAAAGCACAGGCCATTC	102
OC	CCACCGAGACACCATGAGAGC	CCAGCGATGCAAAGTGCG	69
DLX5	CGCTGGGATTGACACAAACAC	GTTACACGCCATTGGGTGCG	131
FGFR1	CCCGCAGCCGCACAT	TGGTATTAACCTCCAGCAGTCTTCAAG	110
FGF2	AGAAGAGCGACCCTCACATCA	GCCAGGGTAACGGTTAGCACAC	91
MSX2	GCCTCGGTCAAGTCGGAAA	CAGGTGGTAGGGCTCGTATGTC	98
NOG	TGCCGAGCGAGATCAAAGG	TGTCTGCGACCACAGCCAC	113
Twist1	ACTTCCTCTACCAGGTCTCTCCAG	CCCTCCATCTCCAGACCG	112
WIF1	CCTCTGTTCAAAGCCTGTCTGC	GTGTCTTCCATGCCAACCTTC	100
18S	TTCGGAAGTGAAGCCATGAT	CGAACCTCCGACTTTCGTTCT	151

QRT-PCR, real-time quantitative reverse-transcriptase polymerase chain reaction.

addition to determining RNA purity by $A_{260}:A_{280}$ ratios using UV spectroscopy.

Real-time quantitative reverse-transcriptase polymerase chain reaction (QRT-PCR)

QRT-PCR was used to determine the differentiation status of serially passaged cells using primers designed, using the Primer Express software, for four common differentiation markers and the reference gene *18S rRNA*. It was also used to validate microarray expression data by measuring the absolute expression levels of selected interesting genes (Table 2). RNA from P4 cells used for microarray validation was extracted from separate flasks from that used for the microarray, effectively comparing biological replicates. All RNA was reverse transcribed into cDNA using SuperScript III (Invitrogen Life Technologies) following the manufacturer's protocol. Absolute quantification was carried out using standard curves covering at least five logs of amplicon copy number, generated by 10-fold serial dilution of target amplicon-containing plasmids (pGEM-T Annandale, Australia). Reactions were carried out using SYBR green (Applied Biosystems, Foster City, CA) in 20 μ l volumes using an ABI Prism 7000 Sequence Detection System (Applied Biosystems) and contained 2 μ l of the cDNA dilutions, and 0.4 μ M of each primer. PCR amplification followed a two-step cycling protocol, 10-min denaturation at 95°C, 40 cycles of 95°C for 15 s, and 60°C for 1 min. Melting curve analysis was conducted to confirm specific amplicon amplification without genomic DNA contamination. Each cell sample and tissue sample reaction was performed in triplicate and standard curve points were performed in duplicate. Absolute copy number values calculated from standard curves were normalized to a calibrator cDNA sample (1 ng was used for RT-PCR) by calculating the ratio of the patient 18S Ct to that of the calibrator sample 18S Ct and expressed as molecules per ng cDNA.

Microarray cDNA synthesis, labeling, hybridization, scanning, and analysis

RNA from two fused suture tissues (Table 1) as well as P4 cells from the same tissue samples was analyzed using Affymetrix Human U133A 2.0 GeneChips. These microarrays contain probe sets covering 18,400 transcripts and variants, including 14,500 well-characterized human genes. Total RNA was prepared for hybridization to the GeneChips following a one-cycle target labeling protocol (Affymetrix GeneChip Expression Analysis Technical Manual, Santa Clara, CA). RNA was reverse transcribed into double-stranded cDNA using SuperScript II (Invitrogen Life Technologies) with a T7 oligomer. Poly(A) RNA spike-in controls were

added along with 2 μ g of tissue or cell total RNA to all cDNA reactions. cDNA was purified with the Affymetrix GeneChip Sample Cleanup Module. Biotin-labeled cRNA was prepared using the Affymetrix GeneChip IVT labeling system, with incubation at 37°C for 16 h. Unincorporated NTPs were removed using the Affymetrix cRNA Sample Cleanup Module and the concentration of cRNA was determined by UV spectroscopy. Unpurified cRNA was run on a 1.5% TBE agarose gel to verify cRNA integrity. 10 μ g of fragmented cRNA was hybridized to each Affymetrix U133A 2.0 GeneChip. Array hybridization, staining and washing were carried out following the manufacturer's protocols using a Fluidics Station 400 (Affymetrix). Arrays were scanned on a GeneChip Scanner 3000 (Affymetrix). CEL intensity files were analyzed in R using Bioconductor packages (www.bioconductor.org). Quality control analyses were carried out following normalization using the probe level model (PLM) algorithm. Normalized unscaled standard error (NUSE) box plots, Mbox plots, and RNA degradation plots were analyzed (Bolstad, 2004) and indicated a high quality of microarray data. For statistical analyses, probe intensity data were normalized using the GCRMA algorithm (Wu et al., 2004). Log₂-fold change versus average log₂ intensity (M versus A) plots were generated for pair-wise whole genome expression comparisons. Genes differentially expressed between fused tissues and P4 cells were identified using the Limma package, which used a linear modeling approach to the data and incorporated an empirical Bayes modification of the standard errors (Smyth, 2004). False discovery rate adjustment of p -values was performed using the Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995). Gene ontology over-representation was analyzed using the GOTree Machine (GOTM) (<http://bioinfo.vanderbilt.edu/gotm/>) normalized to the U133A2.0 gene set (Zhang et al., 2004). Gene Set Enrichment Analysis (GSEA; <http://www.broad.mit.edu/cancer/software/gsea/>) was used to compare probe lists ranked on level of differential expression with gene sets c1-c4 (v2.symbols.gmt) (Subramanian et al., 2005). Gene set exclusion was set at minimum = 4, maximum = 500, with 1,000 weighted permutations executed.

Results and discussion

To create a population of de-differentiated cells, we conducted explant culture of sections of prematurely fused suture tissue. Cells were cultured under minimal medium conditions, which have been shown previously to produce a population mostly composed of cells characteristic of preosteoblasts (de Pollack et al., 1997), and were passaged four times (P4) to ensure that any residual tissue-related expression proposed to exist in P0 and

P1 cells was lost. Cells from a later passage were not used as it has been shown that bone cells after passage 6 show a loss of osteoblastic properties, measured as a responsiveness to osteoinductive factors (Marie et al., 1989). Previously, P4 human and rat calvarial cells have been observed to reinitiate expression of osteoblastic markers and mineralize under differentiating conditions, indicating no loss of osteoblastic potential (Owen et al., 1990; Ratisoontorn et al., 2005). We validated our novel *in vivo-in vitro* approach by showing that similar patterns of differential gene expression are seen for an *in vivo-in vitro* comparison between unfused and prematurely fused suture tissues. We also show that de-differentiation is achieved from P1.

Verification of explant cell de-differentiation

The basis of our experimental approach was the identification of differential expression between an undifferentiated population of cells that were "de-differentiated" by culture in minimal medium and the differentiated tissue from which they were isolated. To verify de-differentiation, we analyzed the expression of four common osteoblast differentiation markers. *Collagen type 1 alpha 1* (*COL1A1*) is an early marker of osteoprogenitor cells; *alkaline phosphatase* (*ALP*) is a marker of osteoprogenitors/preosteoblasts and has maximal expression during matrix maturation; *bone sialoprotein* (*BSP*) is expressed transiently early in osteoprogenitors and then up-regulated in mineralizing osteoblasts; and *OC* is expressed by mature mineralizing osteoblasts (Liu et al., 2003). Figure 1 shows that over successive passages in minimal medium, cells isolated from fused sutures show only a slight decrease in gene expression of the early osteoblastic markers, while there is a dramatic 1,000-fold decrease in *BSP* and *OC* expression at P0 and a further decrease at P1 that is maintained through to P3. The decrease in *BSP* and *OC* expression suggests that the cells were equivalent to undifferentiated osteoblasts, such as osteoprogenitors or preosteoblasts.

It is possible that such an expression pattern may also indicate the considerable selection of fibroblasts in cul-

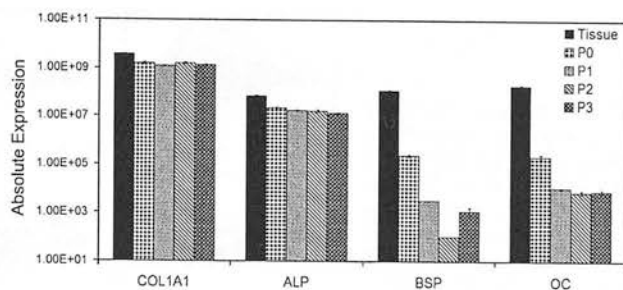


Fig. 1 De-differentiation was observed in serially passed fused suture cells grown in minimal medium. QRT-PCR expression of mature (*BSP* and *OC*), but not immature (*COL1A1* and *ALP*), osteoblast markers dramatically decreased after explant culture (P0–P3) compared with *in vivo* tissue expression. Absolute expression values represent molecules per ng cDNA. Error bars represent triplicate QRT-PCR analyses for patient #58 fused sagittal samples. *BSP*, bone sialoprotein; *OC*, osteocalcin; *COL1A1*, collagen type 1 alpha 1; *ALP*, alkaline phosphatase; QRT-PCR, real-time quantitative reverse-transcriptase polymerase chain reaction.

ture that do express *COL1A1* and *ALP*, but not *BSP* or *OC*. To further confirm the selection of osteoblastic rather than fibroblastic cells, P4 cells were grown in osteogenic medium and analyzed for the formation of a bone-like matrix. After 16 days, cells had formed a collagenous mineralized matrix (Fig. 2A), a specific function of osteoblasts but not fibroblasts (Ecarot-Charrier et al., 1983; Nefussi et al., 1985), while cells grown in minimal medium remained unmineralized (Fig. 2B). Furthermore, cells grown in osteogenic medium exhibited a significant increase in *ALP*, *BSP*, and *OC* expression compared with cells grown in minimal medium (Fig. 2C). This confirmed the selection of a cell population made up predominantly of preosteoblastic rather than fibroblastic cells.

Microarray analysis of genes differentially regulated in prematurely fused suture tissue

Pairwise comparisons of fold change (*M*) to average signal intensity (*A*) were conducted for all four samples

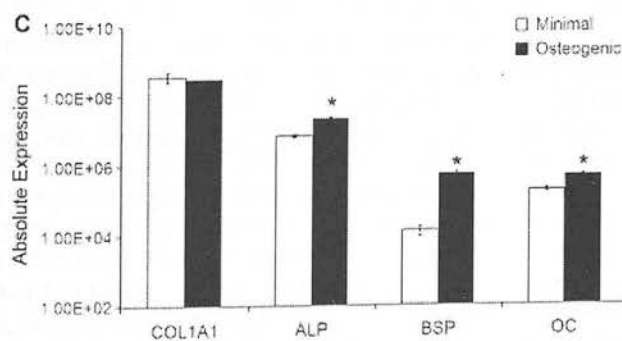
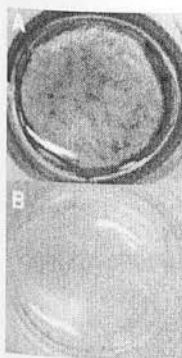


Fig. 2 Cultured suture cells are osteoblastic. (A) Passage 4 cells grown in osteogenic medium for 16 days stain positive for the formation of a collagenous matrix (van Gieson stain, pink/orange) and form mineralized bone nodules (von Kossa stain, black) indicative of osteoblastic cells. (B) Cells grown in minimal medium do not form a collagenous mineralized matrix. (C) Cells grown in osteogenic medium have significantly (*, $p < 0.05$) increased expression of osteoblastic markers *ALP*, *BSP*, and *OC*, compared with those in minimal medium, indicating that cells are differentiating into mature mineralized osteoblasts. Absolute expression values represent molecules per ng cDNA. *BSP*, bone sialoprotein; *OC*, osteocalcin; *ALP*, alkaline phosphatase.

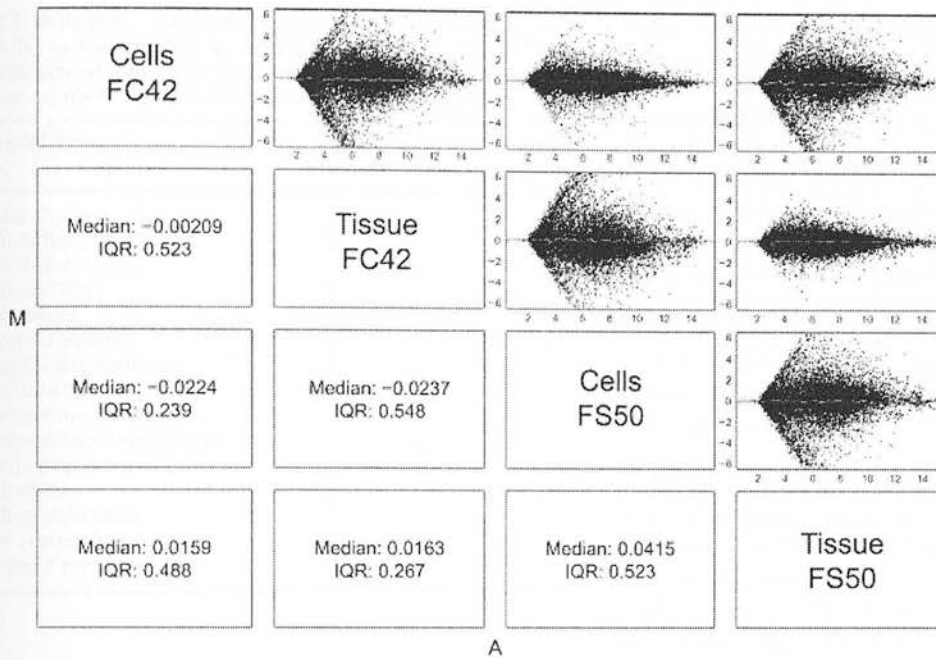


Fig. 3 M versus A plots for each combination of tissue or cultured cells from sagittal or coronal sutures where M for each gene is the \log_2 -fold change (intensity ratio) between the two samples and A is the mean \log_2 intensity of the two samples. In all cases, gene data-points concentrate around $M=0$ for all A , indicating no systematic differences between the arrays. However, while data-points in tissue-tissue and cell-cell comparisons cluster tightly around $M=0$, many more genes are differentially expressed in tissue-cell comparisons (large positive or negative M values). F, fused; C, coronal; S, sagittal; 42 and 50, patient IDs.

in M versus A plots (Fig. 3). The two tissue samples showed the least variation in gene expression, followed by the two cell samples. This indicates that there was little variation in gene expression between patients and in culture conditions. Importantly, there were extensive differences in expression between tissue samples and their corresponding de-differentiated cells. Differential expression analysis of average fused tissue versus average P4 cell intensity identified 1,407 probe sets showing significantly ($p < 0.05$) different expression. Of these, 508 were >10 -fold, 235 were >100 -fold, and 22 were $>1,000$ -fold differentially expressed.

Although calvarial sutures are composed mostly of osteoblastic cells, they also include hematopoietic cells, endothelial cells, osteoclasts, fibroblasts, and adipocytes. Thus, differentially expressed genes will not solely be involved in osteoblastic differentiation but will also include those involved in suture morphogenesis more generally. During suture morphogenesis, cell-cell interactions between the different cell types are fundamental to the correct control of suture morphogenesis (Opperman, 2000; Ogle et al., 2004). For example, osteoblasts are known to excrete angiogenic factors to promote vascularization within the suture due to the high oxygen requirement of the tissue, and angiogenic cytokines have been suggested as important controllers of suture fusion (Song et al., 2004; Kacena et al., 2006a), while megakaryocytes within bone are known to excrete osteogenic factors and inhibit osteoclastogenesis (Kacena and Horowitz, 2006; Kacena et al., 2006b). Moreover, the dura mater that underlies the suture excretes soluble factors into the suture region to control suture patency and/or fusion (Opperman et al., 1995; Bradley et al., 1997; Levine et al., 1998). Thus, one of

the significant advantages of our approach is that we are comparing expression between a cellular monolayer culture with that of a tissue complex. Consequently, genes will be identified that are involved in a variety of processes within the tissues that are controlled by different cell types that are fundamental to suture morphogenesis and potentially initiating suture fusion. The stark contrast between these two sample types is likely to enhance the ability to identify differential gene expression patterns in distinction to a comparison between two complex tissues comprised of cells of varying degrees of differentiation. Through a thorough analysis of the biological functions of the differentially expressed genes, the likely cell types expressing those genes and the processes they are involved in can be inferred, leading to a more comprehensive understanding of the regulation of human suture fusion. Furthermore, the comparison between *in vitro* and *in vivo* expression will identify genes that are de-regulated when cells are grown in a monolayer compared with when in the three-dimensional architecture of the *in vivo* ECM. Thus, this comparison has the potential to identify gene products that can be used as growth medium supplements to induce *in vivo*-like expression/differentiation in an *in vitro* situation.

To evaluate that functional significance of the large number of identified differentially expressed genes, gene ontology (GO) over-representation analysis was carried out for the top 200 up-regulated and 200 down-regulated probe sets. Over-represented biological functions included skeletal development, tissue remodeling, hemopoietic development, vascular development, cell differentiation, and proliferation (Table 3). These are all processes regulated during suture morphogenesis and support the relevance of the identified genes. These

Table 3 Biological processes significantly over-represented ($p < 0.01$) in the top 200 up- and 200 down-regulated probe sets between prematurely fused sutures relative to de-differentiated cells, and the number of genes associated with each process

Biological Process	Higher in tissue	Lower in tissue
Cellular process		
Cell adhesion	17	19
Cell differentiation	13	12
Cell motility	11	9
Development		
Morphogenesis	9	16
Skeletal development	7	5
Ossification	4	2
Vasculature development	1	5
Hemopoietic development	11	1
Physiological process		
Cell death	10	15
Cell proliferation	9	15
Tissue remodeling	4	2
Immune response	55	10

biological processes also indicate the likely cell types within the suture expressing these genes. Interestingly, a number of genes typically associated with an immune response were also significantly over-represented. Some of these (*CD36*, *CD37*, *CD44*, *CD48*, *CD53*, *CD163*, and *CXCR4*) have been identified previously in mouse calvaria primary osteoblasts *in vitro* (Roman-Roman et al., 2003). The expression of immune genes may represent osteoclastogenesis and the highly vascular nature of suture tissue. However, it is also possible that an immune response plays a functional role during suture fusion, as it has been suggested previously that subpopulations of osteoblasts isolated from human mandibles may have immune functions in bone (Reyes-Botella et al., 2000).

To further clarify the biological significance of the most differentially expressed genes, Gene Set Enrichment Analysis (GSEA) was conducted. A ranked list of the same probe sets analyzed by GOTM was compared with the four collections of gene sets within the MSigDB catalogue: cytogenetic, functional, regulatory-motifs, and expression neighborhoods. Gene sets that significantly correlated with genes with increased expression in de-differentiated cells included genes up-regulated in stem cells, genes down-regulated in fibroblasts upon serum exposure, genes regulated by the transcription factors, serum-response factor (SRF) and Yin Yang 1 (YY1), genes up-regulated by TGF- β and TNF- α , and genes that are down-regulated by cytomegalovirus infection (supplementary Fig. S1A). The former gene sets verify the progenitor-like nature of the de-differentiated cells and their removal from the highly vascular *in vivo* environment, while the latter gene set reflects the lower expression of genes involved in an immune response in the de-differentiated

cells. Conversely, immune response-activated gene sets were the most significantly correlated to those genes with increased expression in the fused suture tissues (supplementary Fig. S1B). This included genes up-regulated in dental caries, genes expressed during viral clearance, during neutrophil differentiation, and during T-cell differentiation and activation. Leading edge analysis of these highly correlated gene sets identified two subsets of genes that were shared between gene sets (supplementary Fig. S1C). The first was between gene sets linked to an infection phenotype and the second to neutrophil and lymphocyte differentiation. These results further highlight a potential link between premature suture fusion and immune response activation.

Microarray data were then investigated at the gene level. A selection of genes with the greatest differential expression is shown in Table 4. The gene showing the highest level of differential expression was *defensin alpha 1* (*DEFA1*, 6,982-fold higher in fused tissue). It is involved in immune response and epithelial wound closure through increased cell migration (Aarbiou et al., 2004). Given this function, we speculate that it may also play a role in premature suture closure. Another immune response gene with higher expression in fused suture tissue was *CD24* (233-fold). In epithelial cells, down-regulation of *CD24* is associated with up-regulation of *TWIST1* and *TGFB3* (Ye et al., 2006). Both of these genes are expressed in suture mesenchyme and their expression inhibits osteoblast differentiation and maintains suture patency (Roth et al., 1997; Rice et al., 2000; Opperman et al., 2002b; Bialek et al., 2004). *CD24* could therefore play a role in modulating expression of genes that regulate osteoblast differentiation and suture morphogenesis. *CD24* is also a negative regulator of cell migration and signaling via the chemokine receptor *CXCR4* (Schabath et al., 2006), which we found to have higher expression in de-differentiated cells compared with fused suture tissue (741-fold). There were also a large number of hemoglobin genes with higher expression in fused suture tissue, consistent with the vascularity of the tissue compared with the monolayer culture, further validating the microarray results.

A number of osteogenic genes were also highly differentially expressed between fused suture tissue and de-differentiated cells (Table 4). *Osteomodulin* (*OMD*, 557-fold higher in fused tissue) is a small leucine rich-repeat proteoglycan (SLRP) that can be induced by BMP2 and is an early marker for terminally differentiated osteoblasts (Rehn et al., 2006). *WNT inhibitory factor 1* (*WIF1*, 239-fold higher in fused tissue) was recently found to be essential in controlling terminal differentiation in cultured murine cranial osteoblasts by inhibiting canonical Wnt signaling (Vaes et al., 2005). Furthermore, in an *in vivo* analysis *WIF1* was increased in mouse parietal bone in comparison with suture mesenchyme (Cho et al.,

Table 4 Top 50 genes with greatest significance in differential expression ($p < 0.05$) between fused suture tissue and de-differentiated cells

Gene	Affymetrix probe ID	Gene Bank Number	Gene description	Fold Change in Tissues
DEFA1	205033_s_at	M26602	Defensin α 1, myeloid-related sequence	6982.12
HBB	217232_x_at	NM_000518	Hemoglobin β	6252.06
HBA1	214414_x_at	NM_000517	Hemoglobin α 1	5823.21
S100A8	202917_s_at	NM_002964	S100 calcium binding protein A8 (calgranulin A)	4913.00
BGLAP	206956_at	NM_007541.1	Bone γ -carboxyglutamate (gla) protein (osteocalcin)	3713.07
HBG2	213515_x_at	NM_000184	Hemoglobin γ G	1437.39
PRG2	211743_s_at	NM_002728	Proteoglycan 2, bone marrow (natural killer cell activator)	1151.94
IBSP	207370_at	L20232.1	Integrin-binding sialoprotein (bone sialoprotein)	851.58
FABP4	203980_at	NM_001442	Fatty acid binding protein 4, adipocyte	599.28
DSP	200606_at	NM_004415	Desmoplakin	- 594.02
DMP1	208175_s_at	NM_004407	Dentin matrix acidic phosphoprotein	585.31
OMD	205907_s_at	NM_005014	Osteomodulin	556.88
MNDA	204959_at	NM_002432	Myeloid cell nuclear differentiation antigen	505.00
CYBB	203923_s_at	NM_000397	Cytochrome b-245, β polypeptide	347.42
PTPRZ1	204469_at	NM_002851	Protein tyrosine phosphatase, receptor-type, Z polypeptide 1	275.72
MFAP5	209758_s_at	NM_003480	Microfibrillar associated protein 5	- 263.39
PTPRC	212588_at	NM_002838	Protein tyrosine phosphatase, receptor type, C	247.75
WIF1	204712_at	NM_007191	WNT inhibitory factor 1	239.36
OLFM4	212768_s_at	NM_006418	Olfactomedin 4	234.51
CD24	266_s_at	BC007674	CD24 antigen (small cell lung carcinoma cluster 4 antigen)	233.79
MS4A3	210254_at	NM_006138	Membrane-spanning 4-domains, subfamily A, member 3	230.97
CRISP3	207802_at	NM_006061	Cysteine-rich secretory protein 3	200.44
LCP1	208885_at	NM_002298	Lymphocyte cytosolic protein 1 (L-plastin)	187.84
ALOX5	204446_s_at	NM_000698	Arachidonate 5-lipoxygenase	180.56
FGFR3	204379_s_at	BB732903	Fibroblast growth factor receptor 3	169.69
CA1	205950_s_at	BC027890	Carbonic anhydrase I	154.70
LEF1	221558_s_at	NM_016269	Lymphoid enhancer-binding factor 1	153.26
DPT	213071_at	NM_001937	Dermatopontin	145.50
FCN1	205237_at	NM_002003	Ficolin (collagen/fibrinogen domain containing) 1	137.55
CEACAM6	211657_at	NM_002483	Carcinoembryonic antigen-related cell adhesion molecule 6	136.31
SPOCK	202363_at	NM_004598	Sparc/osteonectin, cwcv and kazal-like domains proteoglycan	- 128.84
MMP8	207329_at	NM_002424	Matrix metalloproteinase 8 (neutrophil collagenase)	117.42
NQO1	201467_s_at	BC007659	NAD(P)H dehydrogenase, quinone 1	- 113.61
IGL2	215121_x_at	AA680302	Immunoglobulin lambda constant 2	92.18
ARG1	206177_s_at	NM_000045	Arginase, liver	90.24
SORL1	212560_at	NM_003105	Sortilin-related receptor, L(DLR class) A repeats-containing	90.19
IRF4	204562_at	NM_00246	Interferon regulatory factor 4	81.13
RGS1	216834_at	NM_002922	Regulator of G-protein signalling 1	72.92
GCA	203765_at	NM_012198	Grancalcin, EF-hand calcium binding protein	72.33
RAG1	206591_at	NM_000448	Recombination activating gene 1	68.07
SATB2	215591_at	AK025127	SATB family member 2	63.86
RNASE6	213566_at	NM_005615	Ribonuclease, RNase A family, k6	61.76
IQGAP2	203474_at	NM_006633	IQ motif containing GTPase activating protein 2	61.57
ARHGAP15	218870_at	NM_018460	Rho GTPase activating protein 15	58.03
ALAS2	211560_s_at	NM_000032	Aminolevulinatase, delta-, synthase 2	53.73
STC2	203439_s_at	NM_003714	Stanniocalcin 2	- 51.69
IGSF3	202421_at	NM_001542	Immunoglobulin superfamily, member 3	48.91
CD163	203645_s_at	NM_004244	CD163 antigen	47.51
MS4A4A	219607_s_at	NM_024021	Membrane-spanning 4-domains, subfamily A, member 4	45.40
GARP	203835_at	NM_005512	Leucine-rich repeat containing 32	- 36.84

2006). Our results are in line with these studies and we thus suggest that *WIF1* is involved in late-stage osteoblast differentiation in human calvarial sutures. We also identified up-regulation of *MMP13* (733-fold), *MMP9* (390-fold), *DMP1* (585-fold), and *PTPRZ1* (276-fold) in mineralized tissues, which is also consistent with the aforementioned *in vivo* mouse study (Cho et al., 2006). Therefore, our *in vivo-in vitro* approach successfully identifies genes involved in an *in vivo* differentiated phenotype.

Significant changes in the expression of additional genes in the Wnt signaling pathway were also noted in prematurely fused sutures (Tables 4 and 5). Canonical Wnt signaling stimulates osteogenesis through multiple mechanisms (for a review, see Krishnan et al., 2006) whereas non-canonical signaling seems to be linked to prolonged survival of cells via inhibition of apoptosis (Almeida et al., 2005). Decreased expression of canonical signaling gene *WISP2* (25-fold) and non-canonical signaling genes *WNT5A* (20-fold) and *frizzled 2*

Table 5 Expression of bone related genes in tissue versus de-differentiated cells

Gene name	Accession No.	Fold change	
		Up	Down
(1) Bone differentiation markers			
Collagen type I	NM_000088	2.08	
Alkaline phosphatase	NM_000478	2.12	
Bone sialoprotein	L20232.1	851.58	
Osteopontin	NM_009263.1	2226.25	
Osteocalcin	NM_007541.1	3713.07	
Osteonectin	NM_003118	1.68	
(2) Transcription factors			
DLX5	NM_010056	6.91	
DLX6	AF022078.1		1.01
MSX1	BC016426.1		2.34
MSX2	NM_013601.1		8.13
RUNX2	D14636.1	1.06	
TWIST	NM_011658.1	1.26	
(3) Bone signaling genes			
β-Catenin	BI134907	1.33	
BMP2	AV239587	20.88	
BMP4	NM_001202	1.20	
BMP7	NM_001719	1.08	
DKK1	NM_010051.1	2.43	
DKK2	NM_020265.1		1.04
DKK3	NM_015881		4.55
EGFR	NM_005228		16.04
FGF2	NM_002006		59.43
FGF4	NM_002007		1.02
FGF7	NM_008008		15.12
FGFR1	M33760.1	6.04	
FGFR2	NM_010207	7.71	
FGFR3	BB732903	169.69	
FZD2	NM_020510.1		32.91
IHH	BC034757	1.01	
LEF1	NM_016269	153.26	
LRP5	NM_002335		1.28
OGN	NM_014057	83.61	
OMD	NM_005014	556.88	
PTHR1	BC031578	69.01	
TGFB1	NM_011577.1		1.28
TGFB2	NM_003238		1.58
TGFB3	NM_003239		1.34
TGFBR1	NM_004612		2.71
TGFBR2	NM_001024847	3.27	
WNT2	BC026373.1		1.01
WNT5A	NM_003392		23.65
WISP2	NM_003881		26.94
WIF1	NM_007191	239.36	
(4) Bone remodeling genes			
MEPE	NM_020203	388.24	
MMP8	NM_002424	117.42	
MMP9	NM_004994	389.95	
MMP13	NM_002427	733.34	
(5) Apoptosis-related genes			
CASP1	NM_033292	21.27	
PAWR	NM_002583		10.28
PERP	NM_022121		24.27
BID	NM_001196	1.14	
GAS6	NM_000820		6.06
TNF	NM_000594		1.01
(6) Cell Cycle-related genes			
PCNA	NM_002592	2.72	
CCND1	NM_053056		8.49
P21/CIP1/CDKN1A	NM_000389		21.21
RARRES1	NM_002888	51.31	
TP53	NM_000546		2.55

(30-fold) point to decreased activity of both Wnt signaling pathways in fused sutures. This is consistent with the observed increased expression of *WIF1*. Our *in vivo-in vitro* observations confirm the recent identification of the down-regulation of Wnt signaling genes by *FGFR2* mutations causal for craniosynostosis in transgenic mouse osteoblastic cells (Mansukhani et al., 2005).

However, the canonical Wnt/ β -catenin signaling transcription factor *Lymphoid enhancer-binding factor 1* (*LEF1*, 153-fold) was also identified to be up-regulated in differentiated calvarial tissue. This transcription factor binds the transcriptional coactivator β -catenin within the nucleus to form the penultimate downstream mediator of the Wnt signaling pathway (Novak and Dedhar, 1999), one role of which is to promote osteoblast differentiation (Bain et al., 2003). Recently, it has been found that *Lef1* is down-regulated in developing bones of embryonic *Runx2* homozygous null mice in which osteoblasts fail to differentiate (James et al., 2006) as *Runx2* is an activator of osteoblast differentiation (Ducy et al., 1997). Thus, *LEF1* may be involved in inducing osteoblast differentiation in human calvarial sutures via either canonical Wnt signaling-mediated or *RUNX2*-mediated mechanisms.

The nuclear matrix protein *SATB* family member 2 (*SATB2*) has recently been shown to act as a molecular node in a transcriptional network regulating skeletal development and osteoblast differentiation (Dobrev et al., 2006). We identified a 62-fold up-regulation of *SATB2* in prematurely fused calvarial suture tissue compared with de-differentiated cells. This is consistent with the study by Cho et al. (2006), which identified an increased expression of *Satb2* in mouse parietal bones compared with suture mesenchyme. *SATB2* directly interacts with, and enhances, the activity of *Runx2* to promote osteoblast differentiation (Dobrev et al., 2006). However, its expression may also be partly controlled via *RUNX2* as calvarial bones of *Runx2*^{-/-} mice have decreased *Satb2* expression (Vaes et al., 2006). Individuals with a translocation at 2q32-q33 that interrupts the *SATB2* gene develop a cleft palate (Fitzpatrick et al., 2003). *Satb2* haploinsufficient mice develop a cleft palate and abnormalities in jaw formation (Britanova et al., 2006). Together with these observations, the identification of increased *SATB2* expression in human calvarial tissue suggests a fundamental role for this protein in human suture morphogenesis and potentially premature suture fusion.

Retinoic acid (RA) is a known craniosynostosis-causing teratogen (Yip et al., 1980; Morriss-Kay, 1993). We identified up-regulation of the retinoic acid receptor responder (tazarotene induced) 1 gene (*RARRES1*, 51-fold; Table 5) in prematurely fused suture calvarial tissue. RA stimulates differentiation of murine osteoprogenitor cells and inhibits proliferation (Nagasawa et al., 2005; Song et al., 2005). *RARRES1* is a type I membrane

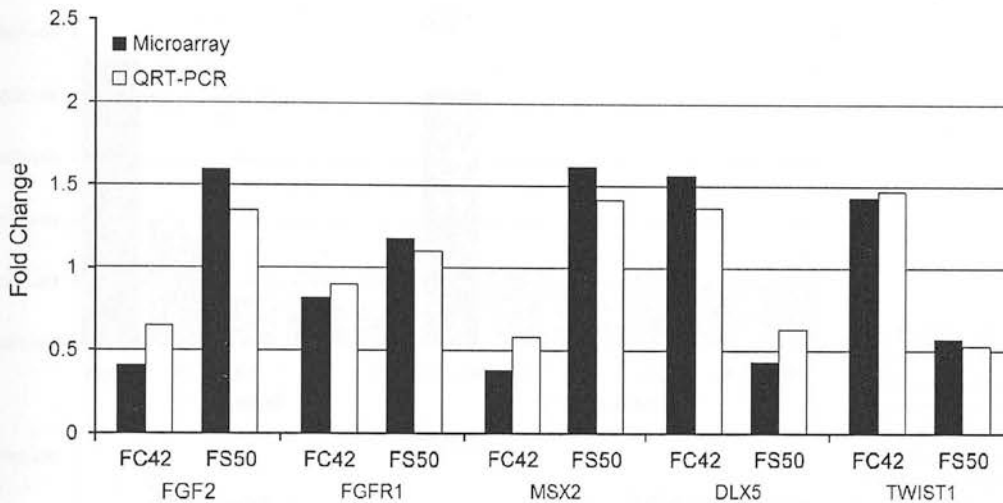


Fig. 4 Microarray validation by QRT-PCR. Expression values for five genes were analyzed in P4 cells isolated from two fused suture tissue samples (FC42 and FS50). Two biological replicates of P4 cells were isolated from each tissue sample: one was analyzed by QRT-PCR and one by microarray. Fold change was calculated separately for each analysis technique as a ratio of patient sample expression to combined-patient sample mean expression for each gene. Fold change estimates from microarray data were comparable with that estimated

for biological replicates by QRT-PCR, with the greatest fold change difference between the two platforms being 0.3. F, fused; C, coronal; S, sagittal; 42 and 50, patient IDs; QRT-PCR, real-time quantitative reverse-transcriptase polymerase chain reaction.

protein that is up-regulated by retinoic acid receptor signaling (Nagpal et al., 1996). Thus, the observation of increased expression of the RA responder gene *RARRES1* within prematurely fused sutures suggests a mechanism that facilitates an increased rate of differentiation of calvarial suture osteoblastic cells resulting in premature suture fusion.

Among those genes differentially expressed were also a large number of those genes known to be fundamental to the processes of suture morphogenesis and/or osteogenesis (Table 5). This further verifies the ability of our *in vitro-in vivo* approach to identify genes involved in the *in vivo* process of suture fusion and morphogenesis. There was a dramatic up-regulation of markers of mature osteoblasts (*OC*, 3,713-fold; *OP*, 2,226-fold; and *BSP*, 852-fold), and only a 2-fold change in expression of osteoblast progenitor markers (*COL1A1* and *ALP*) in the tissue, confirming the results in Figure 1. Interestingly, while there was a dramatic increase in *OC* expression, there was no difference in the expression of *RUNX2*, a primary transcription factor of *OC* (Ducy et al., 1997). This may be explained by the fact that *MSX2*, an antagonist of *RUNX2*, is down-regulated in the tissue and an agonist, *DLX5*, was up-regulated (Shirakabe et al., 2001), thus potentiating the effect of *RUNX2* on transcriptional activation of *OC*. It is therefore possible that *OC* expression is controlled through the regulation of expression of *RUNX2* modifiers, rather than directly through the regulation of *RUNX2* expression. The functional activation of *RUNX2* is also supported through our observation of increased expression of *OMD* and *osteolectin* (84-fold), two SLPRs that share a *RUNX2*-binding domain (Tasheva et al., 2004).

Fibroblast growth factor 2 (FGF2) was decreased 60-fold in prematurely fused sutures (Table 5). *FGF2* stimulates the differentiation of osteogenic precursors but inhibits the expression of a mature osteogenic phe-

notype by down-regulating *ALP*, *COL1A1*, and *OP* and blocking mineralization in less mature cells (Debiais et al., 1998; Kalajzic et al., 2003; Fakhry et al., 2005). This suggests that *FGF2* is down-regulated in the fused suture tissue to allow late-stage osteoblast differentiation. However, *FGF2* has also been shown to stimulate its own expression abnormally *in vitro* (Hurley et al., 1994). This *in vitro* phenomenon could therefore contribute to the relatively large up-regulation seen in the cultured cells compared with fused suture tissue.

Among those genes expressed in suture mesenchyme (for review, see Rice, 2005), *MSX2* was down-regulated 8-fold in fused tissue, while *TWIST1* was unaffected (Table 5). Conversely, *FGFR1* and *DLX5*, which are expressed in osteogenic fronts, were correspondingly up-regulated in fused sutures. Interestingly, *FGFR3* was 170-fold up-regulated in prematurely fused sutures but has been identified previously to be only weakly expressed in developing mouse osteogenic fronts (for a review, see Morriss-Kay and Wilkie, 2005). This may indicate a more important role for *FGFR3* in human postnatal development or a greater modulation of this gene *in vitro* compared with the other *FGFRs*.

Differential expression between the two de-differentiated cell samples was also assessed. It was noted that all genes with >4-fold differential expression between the two patients' cells were not significantly differentially expressed in the averaged tissue-cell comparison (supplementary Fig. S2). This suggests that there may be some intrinsic expression in each cell population that is not equally affected by cell culture. These differences may reflect the fact that cells were isolated from individuals with different etiologies of craniosynostosis and that they are from coronal and sagittal sutures. Importantly, however, it shows that only a small portion of genes are differentially expressed between the cell samples and that the vast majority of genes are modulated to the same levels by the culture conditions even for

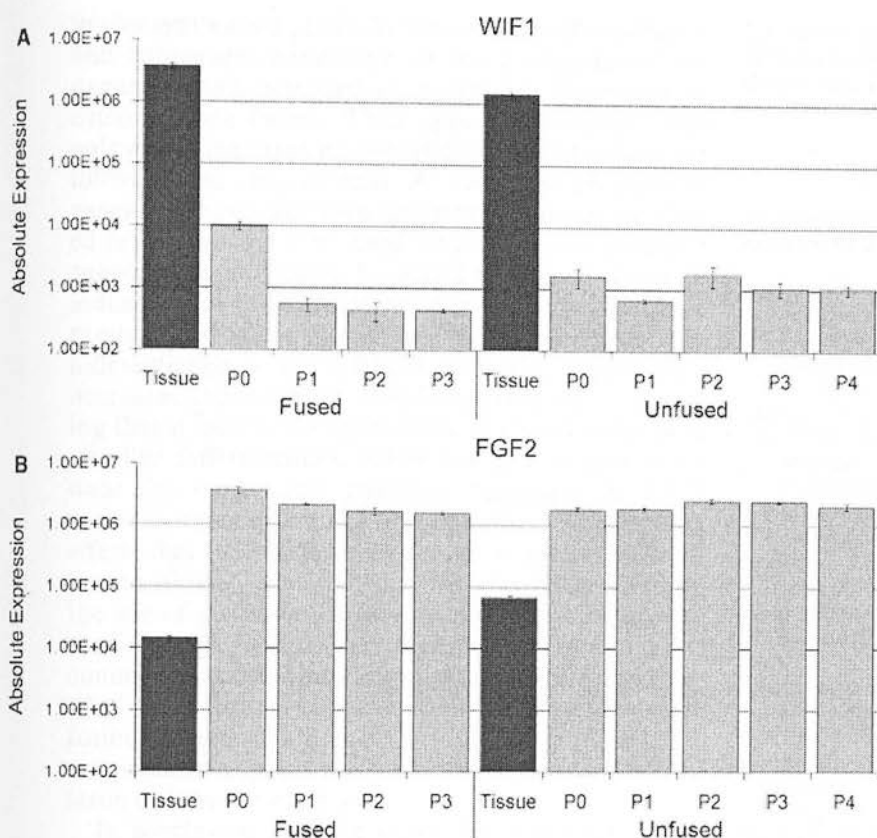


Fig. 5 QRT-PCR absolute expression values for (A) *WIF1* and (B) *FGF2* in fused and unfused suture tissue and corresponding de-differentiated cells (P0–P4) from patient #58. The same pattern of differential expression exists between fused and unfused suture tissue as is seen between fused suture tissue and de-differentiated cells. However, this same pattern of differential expression is only observed between cultured fused and unfused suture cells for one gene, *WIF1*, at P0. After P0, limited difference in the expression of *WIF1* or *FGF2* is observed between de-differentiated cells from fused and unfused sutures. Absolute expression values represent molecules per ng cDNA. *WIF1*, *WNT inhibitory factor 1*; *FGF2*, *fibroblast growth factor 2*; QRT-PCR, real-time quantitative reverse-transcriptase polymerase chain reaction.

samples of different etiologies. Thus, the utilization of samples of both syndromic and non-syndromic etiologies has identified genes commonly involved in both forms of the developmental abnormality.

Microarray validation

Microarray expression data were validated for five genes with known roles in suture morphogenesis: *FGF2*, *TWIST1*, *MSX2*, *FGFR1*, and *DLX5*, using absolute QRT-PCR. Biologically replicated P4 cell samples isolated from the same two tissues, but from flasks different from that used for the microarray experiments, were used for real-time validation experiments. No obvious fold change differences were identified between microarray and QRT-PCR expression estimates for any gene for either patient sample analyzed (Fig. 4).

Validation of comparative approach

To confirm the ability of our *in vivo*–*in vitro* approach to identify genes involved in suture morphogenesis and osteoblastic differentiation, we hypothesized that genes identified as being up- or down-regulated in fused sutures relative to P4 cells should be similarly regulated relative to an unfused suture. It was expected, however, that differences should not be as large because the col-

lected unfused suture tissue contains a mixture of both proliferating and differentiating cells. Accordingly, we chose *WIF1* (up-regulated) and *FGF2* (down-regulated) to analyze in unfused and prematurely fused suture tissue. As expected, both genes were shown to exhibit similar, but less dramatic differential expression patterns when compared with unfused sutures (Fig. 5). *WIF1* had a 2.7-fold increased expression in fused compared with unfused suture tissues, while *FGF2* had 4.8-fold decreased expression in fused compared with unfused suture tissues.

The analysis of serially passaged cells from both tissue types also shows that both genes have a significantly large modulation in expression during culturing in minimal medium compared with their expression *in vivo* (Fig. 5). Furthermore, there is limited difference in gene expression between cultured cells from both fused and unfused suture tissues following P0. This suggests that immediately after first passage, most tissue-specific expression is lost and that essentially from P1 an *in vitro*-specific level of expression is reached irrespective of the differentiation status of the tissue cultured. This phenomenon is further emphasized by the significant up-regulation of *FGF2* to the same extent in both cell populations immediately upon explantation (P0). This dramatic loss of *in vivo* expression immediately following explantation supports the hypothesis by Owen et al. (1990) that removal of cells from their

in vivo synthesized 3D ECM results in de-differentiation and subsequent reinitiation of the developmental sequence is only activated once cells are re-exposed to osteoinductive factors. There appear to be specific signals within the tissue microenvironment that sustain the differentiated state of cells. As such, the products of genes that have decreased expression in de-differentiated cells compared with fused suture tissue are potential supplements that could be added to growth media to induce *in vivo*-like differentiation *in vitro*. One such gene product that is commonly used to induce osteoblastic differentiation *in vitro* is BMP2. This gene had a 21-fold decreased expression in de-differentiated cells, suggesting that it may be an appropriate *in vitro* inducer of *in vivo*-like differentiation. Additionally, strategies to reduce the significantly increased expression of *FGF2* within cultured cells may help to eliminate the inhibiting effects that *FGF2* has on late-stage osteoblast differentiation (Fakhry et al., 2005). This may be achieved by the use of serum-free media rather than using growth media supplemented with fetal bovine serum, as is common practice. Thus, this microarray study has identified genes that may be involved in inducing premature fusion of human sutures, as well as gene products that are potential supplements to induce *in vivo*-like osteoblastic differentiation *in vitro*.

In conclusion, we have shown that immediately following explant culture, cells isolated from both unfused and prematurely fused suture tissue de-differentiate, losing the majority of *in vivo*-related gene expression, and after passage in minimal media, adopt a similar stable *in vitro*-specific expression profile irrespective of the fused-unfused state of the explant suture. Moreover, using microarrays, we have shown that a comparison of *in vivo* expression of prematurely fused cranial sutures with *in vitro* expression of de-differentiated explant cell populations from the same tissues successfully identifies a large number of genes commonly associated with osteoblast differentiation and suture morphogenesis. Furthermore, using this *in vivo*-*in vitro* approach, we have identified a number of genes previously unknown to be involved in human suture morphogenesis. This includes a number of Wnt signaling genes, one of which, *WIF1*, was confirmed to be up-regulated in prematurely fused human suture tissue compared with unfused suture tissue. The novelty of this *in vivo*-*in vitro* approach is that genes can be identified using cell culture that are directly implicated in the *in vivo* process of cellular differentiation and tissue morphogenesis. By using a de-differentiated cell population and comparing it with a heterogeneous tissue, genes involved in multiple processes of *in vivo* differentiation can be identified.

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Supplementary material

The following supplementary material is available for this article online:

Fig. S1: GSEA analysis results. Enrichment score (ES) plots of selected interesting genes sets significantly correlated with the top 200 genes significantly up-regulated in (A) de-differentiated cells (red) and (B) prematurely fused suture tissue (blue). The colour bar depicts phenotype correlation based on ranking metric scores. Black bars represent genes ordered by their ranking within the top 200 up- and down-regulated genes sets between de-differentiated cells and prematurely fused sutures tissue. (C) Leading edge analysis indicates which genes within the leading edge subsets are shared between gene sets. This can reveal a biologically important subset of genes within the correlating gene sets. For 11 gene sets which were significantly correlated with genes with increased expression in prematurely fused suture tissue, there were two obvious subsets of shared genes. TYROBP, CD53, CD74, LAPTM5, and PTPRC were shared by gene sets related to an infection phenotype. LCN2, CAMP, CYBB, MPO, and ELA2 were shared by gene

sets related to neutrophil or lymphocyte differentiation and activation. Expression values are represented as colours, where the range of colours (red, pink, light blue, dark blue) shows the range of differential expression values, where red is highest expression in de-differentiated cells and dark blue is lowest expression in de-differentiated cells (i.e. highest expression in prematurely fused suture tissue).

Fig. S2: Volcano plot of fold change versus \log_2 odds of differential expression between mean expression from prematurely fused tissue and mean expression from de-differentiated cultured cells. Genes observed to be differentially expressed by more than 2-fold are located wide of the green lines while genes 10 times more likely to be differentially expressed than not are located above \log_2 odd = 3.3. Significance levels were adjusted for multiple comparisons by the Benjamini-Hochberg procedure which limits the false discovery rate (fraction of significant results that are false) to 1% (red dots) or 5% (blue dots). In a separate comparison, differential

expression was observed for a number of genes between cultured cells from fused coronal sutures and cultured cells from fused sagittal sutures. All genes that showed a differential expression greater than 4-fold have been indicated on the above tissue-cell plot as aqua dots. It can be seen that none of these are significantly differentially expressed between tissue and de-differentiated cells, indicating that those genes which are significantly differentially expressed between patients are not those which are significantly affected by de-differentiation conditions.

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Research article

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Unravelling the molecular control of calvarial suture fusion in children with craniosynostosis

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Abstract

Background: Craniosynostosis, the premature fusion of calvarial sutures, is a common craniofacial abnormality. Causative mutations in more than 10 genes have been identified, involving fibroblast growth factor, transforming growth factor beta, and Eph/ephrin signalling pathways. Mutations affect each human calvarial suture (coronal, sagittal, metopic, and lambdoid) differently, suggesting different gene expression patterns exist in each human suture. To better understand the molecular control of human suture morphogenesis we used microarray analysis to identify genes differentially expressed during suture fusion in children with craniosynostosis. Expression differences were also analysed between each unfused suture type, between sutures from syndromic and non-syndromic craniosynostosis patients, and between unfused sutures from individuals with and without craniosynostosis.

Results: We identified genes with increased expression in unfused sutures compared to fusing/fused sutures that may be pivotal to the maintenance of suture patency or in controlling early osteoblast differentiation (i.e. *RBP4*, *GPC3*, *CIQTNF3*, *IL11RA*, *PTN*, *POSTN*). In addition, we have identified genes with increased expression in fusing/fused suture tissue that we suggest could have a role in premature suture fusion (i.e. *WIFI*, *ANXA3*, *CYFIP2*). Proteins of two of these genes, glypican 3 and retinol binding protein 4, were investigated by immunohistochemistry and localised to the suture mesenchyme and osteogenic fronts of developing human calvaria, respectively, suggesting novel roles for these proteins in the maintenance of suture patency or in controlling early osteoblast differentiation. We show that there is limited difference in whole genome expression between sutures isolated from patients with syndromic and non-syndromic craniosynostosis and confirmed this by quantitative RT-PCR. Furthermore, distinct expression profiles for each unfused suture type were noted, with the metopic suture being most disparate. Finally, although calvarial bones are generally thought to grow without a cartilage precursor, we

show histologically and by identification of cartilage-specific gene expression that cartilage may be involved in the morphogenesis of lambdoid and posterior sagittal sutures.

Conclusion: This study has provided further insight into the complex signalling network which controls human calvarial suture morphogenesis and craniosynostosis. Identified genes are candidates for targeted therapeutic development and to screen for craniosynostosis-causing mutations.

Background

Calvarial bones form by the proliferation and differentiation of multipotent mesenchymal cells into osteoblasts. This process, known as intramembranous ossification, is distinct from the development of the majority of other bones in the body which form by the ossification of a pre-existing cartilaginous matrix (endochondral ossification). Calvaria first form from a condensation of mesenchyme termed the primary centre of ossification. Mesenchymal cell proliferation and subsequent differentiation into osteoblasts occurs at the margins and the bone grows in a radial fashion until the osteogenic fronts of two calvaria approximate each other and structures called sutures form between the bones [1]. These intervening fibrous sutures act as flexible joints between the developing bones allowing the skull to change shape and grow during development. Maintenance of growth at the osteogenic fronts at the edges of the sutures requires a fine balance between proliferation and differentiation. Additionally, apoptosis has a role ensuring that the two osteogenic fronts remain separated [2]. Disruption of any of these processes can result in the premature fusion of calvarial sutures, known as craniosynostosis.

Craniosynostosis is amongst the most common cranial defects, second only to cleft palate. It occurs in 1 in 2500 live births and can be associated with significant morbidity, including mental retardation, deafness, and blindness, in addition to the significant social stigma associated with craniofacial deformation [3]. The condition may be caused by various genetic mutations, exposure to teratogens such as retinoic acid, mechanical stress, or result from certain metabolic or haematologic disorders [4,5]. Non-syndromic craniosynostosis refers to sporadic suture fusion in the absence of other developmental abnormalities and most commonly affects the sagittal suture. Syndromic craniosynostosis occurs as a result of simple genetic mutations and is accompanied by additional developmental abnormalities particularly involving the limbs [6]. Syndromic forms of craniosynostosis commonly affect the coronal suture but other sutures may be affected depending on the underlying genetic mutation. *FGFR2* mutations are the most common and most severe affecting the coronal, metopic, sagittal, and lambdoid sutures. *FGFR3* mutations affect the coronal and/or metopic sutures. *FGFR1*, *TWIST1* and *EFNB1* mutations

generally affect only the coronal suture. *FNB1* and *TGFBRI* mutations have been associated with synostosis of the sagittal and/or lambdoid sutures, while gain-of-function *MSX2* mutations result in synostosis of the coronal and sagittal sutures (reviewed in [7]).

The large number of genes identified as causal for craniosynostosis suggests that a complex molecular network controls suture morphogenesis in humans. In addition, rodent studies have revealed a role in suture formation for transforming growth factor beta (TGF β) signalling mediated by various bone morphogenetic proteins (BMPs) [8-11]. Targeted functional genetic approaches are slowly unravelling the molecular signalling that controls suture morphogenesis. However, there is also a need for a broad experimental approach aimed at identifying all genes and, subsequently, their associated pathways which are essential to suture morphogenesis.

The different phenotypes induced by the known mutations suggest that distinct molecular pathways may be operating in different sutures. This is particularly evident in the case of the metopic suture which, in humans, normally fuses shortly after birth, while the other sutures remain patent until adulthood. This feature of the metopic suture may be explained by the finding in rodents that the frontal suture (equivalent to the metopic suture in humans) is populated by neural crest derived mesenchyme and separates the frontal bones, also of neural crest origin, while the other sutures are a juxtaposition of neural crest and paraxial mesoderm [12-14]. To understand the mechanisms of the fusion process gene expression profiles between the fusing posterior frontal sutures in mice have been compared to profiles from unfused sagittal and coronal sutures [15-20]. However, given that the signalling pathways controlling suture fusion are likely to differ in sutures derived from different developmental origins, it is unclear what such comparisons tell us about these fusion processes. There is, therefore, a need to study differential gene expression between fused and unfused sutures of the same developmental origin.

Subtle differences in cranial biology also exist between rodents and humans. For example, the rodent model created for the Pro250Arg *FGFR1* mutation, which causes Pfeiffer syndrome, develops synostosis of the frontal, sag-

ittal and coronal sutures [21] whereas in humans this mutation commonly affects only the coronal suture. Furthermore, primary cells cultured from patients with *FGFR2* mutations and mice generated with the same mutations show differing proliferation and differentiation characteristics (reviewed in [22]). These differences emphasise that mechanisms controlling rodent suture morphogenesis do not exactly mimic those occurring in human sutures.

In this study we have analysed global *in vivo* expression differences between fused, fusing, and unfused sutures from patients with craniosynostosis to identify genes which are involved in maintaining suture patency and driving suture fusion within each human suture.

Results

Five patients were recruited to the gene identification stage of this study, one diagnosed with syndromic craniosynostosis (Apert syndrome [MIM 101200]) and four with non-syndromic craniosynostosis (Table 1). Sixteen suture samples were obtained from these patients for microarray analysis; nine from unfused sutures, two from fusing, and five from fused sutures (Fig. 1A). To minimise any age-related changes and to eliminate any sex-related effects on the development of craniosynostosis, sutures were obtained from males aged 3-7 months. The stage of fusion was confirmed by 3D computer tomography (CT), MicroCT, and histological analysis and classified as unfused, fusing, or fused (Fig. 1). We performed microarray analyses on RNA isolated from sutures resected at surgery using the Affymetrix Human U133A 2.0 GeneChip platform containing ~18,000 gene transcripts. Microarray data were initially assessed using a number of quality control measures (Additional files 1, 2, 3). RNA digestion plots indicated that all samples showed a high similarity in RNA quality except for one unfused sagittal sample from patient #36 (US36). NUSE and Mbox plots indicated that this sample had a similar expression intensity compared to other samples but had elevated standard errors. We were initially cautious in our interpretation of any particular difference in expression seen with this sample.

Patient genetic background does not adversely affect gene expression

Hierarchical clustering, based on whole genome expression, showed samples typically grouped according to stage of fusion or suture type, and not solely by patient of origin, indicating no adverse patient-specific genetic background biases existed (Additional file 4). Importantly, sutures from the Apert syndrome patient grouped more closely with similar sutures from other non-syndromic patients, than they did to each other. These similarities in gene expression between syndromic and non-syndromic patients were confirmed with additional syndromic sam-

ples using realtime quantitative RT-PCR (qRT-PCR), as described later. This indicates that patient genetic background does not overly impact on gene expression and it provides proof of principle that the combined analysis of syndromic and non-syndromic patient samples can be applied in the study of general mechanisms of craniosynostosis.

Metopic sutures have different gene expression profiles to other sutures

The neural crest origin of the metopic suture mesenchyme, compared to the predominantly mesodermal origin of the other sutures may result in the metopic suture, exhibiting significantly different expression profiles to the other suture types. We therefore initially analysed differential gene expression between fused and unfused sutures treating metopic sutures separately. Microarray expression data were combined for all unfused ($n = 8$) and all fusing/fused ($n = 6$) sutures, from the sagittal, coronal and lambdoid sutures and differential expression was analysed between the two groups. Initially, a subset of differentially expressed probe sets was selected based on those with a multiple testing corrected $P < 0.1$ ($n = 84$) in order to assess how well the analysis separated the two groups of interest. This minimally-selective P-value was chosen to remove a large number of those genes which were not modulated in the two tissue types. Pair-wise correlation of all samples to an arbitrarily chosen unfused suture sample (#36 non-syndromic, unfused coronal) showed that samples were separated based on stage of fusion using the chosen gene subset, with a gradient of relatedness seen for unfused, fusing, and fused tissues (Fig. 2A). Furthermore, the unfused metopic suture grouped with the fused tissues, suggesting that metopic suture mesenchyme has an expression profile more similar to fused tissue. This result indicated our exclusion of metopic suture samples from statistical analyses of differential expression between unfused and fused samples. All other unfused sutures showed a very high correlation in expression between themselves, whereas fused and fusing sutures were more disparate in expression profiles. This broader distribution of fusing and fused sutures may indicate that they were undergoing pathologic fusion of different aetiologies and/or that they were at different stages of the fusion process. Additionally, there was evidence for suture-specific expression, with the unfused sagittal sutures being slightly less correlated to unfused coronal sutures than were unfused lambdoid sutures. This difference was later analysed by comparing expression solely between each unfused suture type; however by pooling unfused sutures for the initial analyses we were also able to identify those genes commonly involved in morphogenesis of all sutures.

Table 1: Phenotypes of patients, identified causative mutations, fusion state and site of obtained sutures.

Analysis	Patient	Phenotype	Sex	Age (m)	Mutation	Fused Suture	Fusing Suture	Unfused Suture
Microarray	#42	Apert syndrome	M	7	FGFR2 Ser252Trp	Coronal ^a Lambdoid ^a	Metopic ^a	
	#36	Unicoronal synostosis	M	3	No FGFR or TWIST1		Coronal ^a	Coronal ^a Sagittal
	#46	Sagittal synostosis	M	5	No FGFR or TWIST1	Sagittal		Coronal Lambdoid ^a Metopic ^a
	#50	Sagittal synostosis	M	6	No FGFR or TWIST1	Sagittal		Coronal
	#58	Sagittal synostosis	M	7	No FGFR or TWIST1	Sagittal ^a	Sagittal ^b	Sagittal ^a Coronal Lambdoid ^a
Validation	#47	Apert syndrome	F	38	FGFR2 Pro253Arg	Metopic		
	#85	Apert syndrome	F	40	FGFR2 Ser252Trp	Sagittal	Metopic	
	#90	Apert syndrome	F	4	FGFR2 Pro253Arg	Coronal	Coronal ^c	Sagittal Metopic Coronal
	#61	Apert syndrome	M	20	FGFR2 Ser252Trp		Coronal	Coronal
	#92	Muenke syndrome	F	6	FGFR3 Pro250Arg			Coronal
	#104	Saethre-Chotzen syndrome	M	14	TWIST1 c.256_276dup	Sagittal Metopic		
	#49	Metopic synostosis	M	10	No FGFR or TWIST1	Metopic		
	#60	Sagittal synostosis	F	9	No FGFR or TWIST1	Sagittal	Sagittal	
	#63	Sagittal synostosis	M	10	No FGFR or TWIST1	Sagittal	Sagittal	Lambdoid
	#72	Lambdoid synostosis	M	26	No FGFR or TWIST1	Lambdoid		
	#91	Sagittal synostosis	F	5	No FGFR or TWIST1			Metopic
	#94	Metopic synostosis	F	9	No FGFR or TWIST1	Metopic	Metopic	Sagittal
	#95	Unicoronal synostosis	M	5	No FGFR or TWIST1	Coronal		
	#41	Normal – Tessier Cleft	F	91	Not tested			Metopic
	#69	Normal – hydrocephalus	F	4	Not tested			Lambdoid
	#73	Normal – cerebellar tumour	M	9	Not tested			Coronal
	#81	Normal – hydrocephalus	F	2	Not tested			Lambdoid
	#87	Normal – cerebellar tumour	M	57	Not tested			Coronal
	#89	Normal – hydrocephalus	M	1 day	Not tested			Lambdoid
Histology	#3	Apert syndrome	M	4	FGFR2 Ser252Trp	Lambdoid		
	#5	Sagittal synostosis	M	3	No FGFR or TWIST1	Sagittal		
	#80	Metopic synostosis	M	6	No FGFR or TWIST1			Coronal Sagittal Lambdoid (x2) Coronal
	#83	Sagittal synostosis	M	6	No FGFR or TWIST1			Sagittal
	#84	Unicoronal synostosis	F	7	No FGFR or TWIST1	Coronal		

^aRNA sample also used for qRT-PCR validation experiments.

^bSample used for validation experiments, but not microarray analysis.

^cTwo samples of varying degrees of fusion were obtained. The sample which was at the earlier stage of fusion was used for validation qRT-PCR; the sample at a later stage of fusion was used for within patient comparison only.

Hierarchical clustering based on the selected subset of genes clearly showed that suture tissue samples formed two clusters, mirroring two states of fusion: fused/fusing and unfused (Fig. 2B). This suggests that fusing sutures are generally more similar to fused than unfused sutures and that it is appropriate to group them together for analyses. It was also noted that in both plots in Figure 2, sample

US36, despite RNA quality concerns, grouped most closely with the other unfused sagittal suture and thus it was appropriate to include this sample in further analyses.

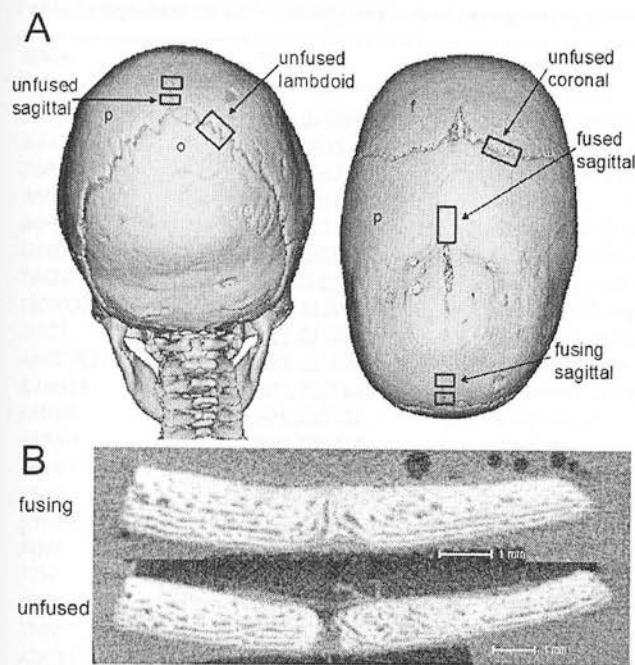


Figure 1
Computer tomography (CT) scans showing site and fusion state of sutures obtained from craniosynostosis patients. A) Posterior and superior (left and right) view of patient #58 indicating where unfused, fusing and fused sutures were obtained from. p, parietal bone; o, occipital bone; f, frontal bone. **B)** MicroCT image demonstrating a fusing and unfused suture. Scale 1 mm.

Genes differentially expressed between fused and unfused sutures

Based on a linear regression analysis of genes differentially expressed between the unfused group of sutures and the group of fusing/fused sutures, 28 genes were significantly (multiple testing corrected $P < 0.05$) differentially expressed (Table 2). Irrespective of P-value, a greater than 2-fold expression difference was found for 829 probe sets; 252 were "increased" and 577 were "decreased" in unfused compared to fusing/fused sutures (Additional file 5). Amongst those genes increased in unfused sutures were *FGFR2*, *TGFB2*, and *epidermal growth factor receptor (EGFR)* (Table 3). All have been previously linked with calvarial development and, in the case of *FGFR2*, with craniosynostosis [23-28]. Thirty two of these 829 probe sets (3.9%), representing 24 genes, had a significant difference in expression (multiple testing corrected $P < 0.05$), suggesting that these are important in the morphogenesis of all sutures. All, except one, were increased in unfused sutures. The identification of such a small number of significantly expressed genes across all suture types is likely due to the fact that different suture types, which may have slightly different gene expression profiles, were combined for the analysis. As we did not want to reject any potentially important genes, including those expressed to varying degrees in different sutures, we carried out further analyses using the genes in the 2-fold list, irrespective of their P-value. Importantly, however, we recognise that those genes with a $P < 0.05$ are more likely to be key regulators of suture morphogenesis, rather than specific to one suture type.

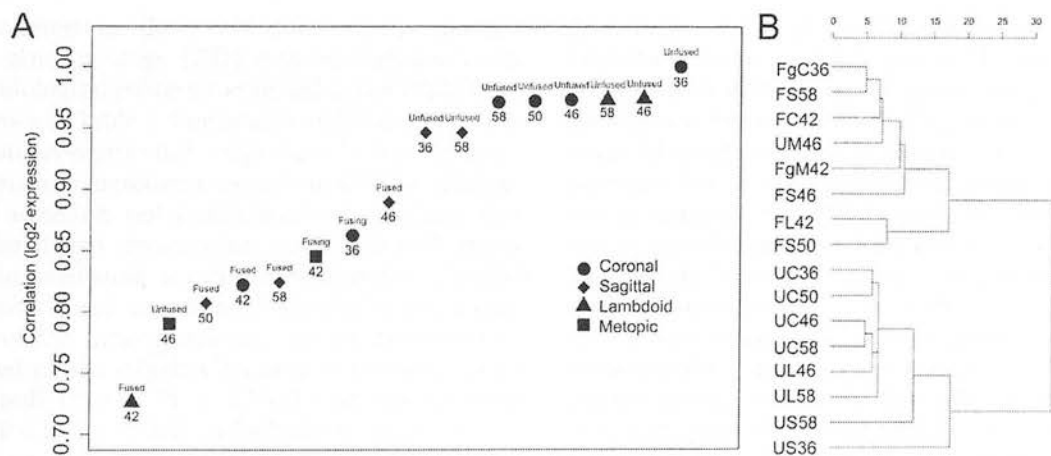


Figure 2
Microarray sample correlations based on a selected gene list (fused v unfused, $P < 0.1$). Correlations are based on genes differentially expressed ($P < 0.1$) between unfused and fused sutures for combined samples from coronal, lambdoid and sagittal sutures. **A)** Correlation to the unfused coronal suture from patient #36 shows a gradient of correlation between unfused, fusing and fused sutures. The unfused metopic suture groups with fused sutures. Patient number is recorded below data points and state of fusion above data points. **B)** Hierarchical clustering separates suture data into unfused and fusing/fused sutures. Unfused lambdoid and coronal sutures are more related to each other than they are to sagittal sutures. U, unfused; Fg, fusing; F, fused; C, coronal; S, sagittal; L, lambdoid; M, metopic; patient number follows sample identifier.

Table 2: Significantly differentially expressed genes: unfused v fusing/fused sutures.

Gene	GenBank	Name	Fold
MFAP4	NM_002404	microfibrillar-associated protein 4	16.50
IL11RA	NM_004512	interleukin 11 receptor, alpha	5.90
RBP4	NM_006744	plasma retinol binding protein 4	37.38
AMPH	NM_001635	amphiphysin	8.27
INHBA	NM_002192	inhibin, beta A (activin A, activin AB alpha polypeptide)	6.94
CIQTNF3	BC016021	CIq and tumor necrosis factor related protein 3	20.25
PRELP	BC032498	proline arginine-rich end leucine-rich repeat protein	10.65
HDHD1A	NM_012080	haloacid dehalogenase-like hydrolase domain containing 1A	-1.78
AGC1	NM_013227	aggrecan 1 (chondroitin sulfate proteoglycan 1)	6.16
ANGPTL2	NM_012098	angiopoietin-like 2	6.67
C1orf24	NM_052966	chromosome 1 open reading frame 24	2.04
FMOD	NM_002023	fibromodulin	5.41
OLFM1	NM_006334	olfactomedin 1	2.55
FBLN1	NM_006486	fibulin 1	12.88
SSPN	NM_005086	sarcospan	4.40
CYFIP2	BC026892	cytoplasmic FMR1 interacting protein 2	-2.68
MNI	NM_002430	meningioma 1	3.72
PTN	NM_002825	pleiotrophin (osteoblast-specific factor 1)	6.25
EGFR	NM_005228	epidermal growth factor receptor	2.71
TNN	NM_022093	tenascin N	6.67
ADCY2	NM_020546	adenylate cyclase 2 (brain)	2.96
PDZRN3	NM_015009	PDZ domain containing RING finger 3	3.71
SPON1	BC041974	spondin 1, extracellular matrix protein	4.73
APP	NM_000484	amyloid beta (A4) precursor protein	1.83
AUTS2	NM_015570	autism susceptibility candidate 2	1.94
GPC3	NM_004484	glypican 3	7.13
HAPLN1	NM_001884	hyaluronan and proteoglycan link protein 1	7.10
LHX6	NM_014368	LIM homeobox 6	-1.80

Twenty-eight genes were identified as being significantly (false discovery rate adjusted $P < 0.05$) differentially expressed in unfused sutures compared to fused/fusing sutures. Genes are listed in order of significance with fold change relative to unfused sutures.

To further categorise the 2-fold differentially-expressed gene list, gene ontology (GO) over-representation was analysed. Biological processes enriched in the 2-fold gene list are shown in Table 4. Genes with higher expression in unfused sutures were found to significantly over-represent processes such as mesoderm formation, skeletal development, cell adhesion, cell surface receptor signalling, and extracellular matrix organisation, consistent with genes involved in regulating suture morphogenesis. Surprisingly, we also noted an extremely significant over-representation within those genes with higher expression in fusing/fused sutures of genes involved in the response to biotic stimuli ($P = 5.73 \times 10^{-41}$) and the immune response ($P = 1.61 \times 10^{-34}$). As fold change is not the only useful characteristic, GO over-representation analysis was also conducted irrespective of fold change for all probe sets with a minimally selective $P < 0.25$ ($n = 261$) and similar categories were identified.

Gene Set Enrichment Analysis (GSEA) was then used to assess the significance of this set of differentially expressed genes at the molecular level. The ranked list of 2-fold differentially expressed genes was compared with a curated

database consisting of molecular pathways and publicly available microarray experiments (Additional file 6). Such a comparison identifies which molecular pathways share a group of genes with our identified gene list, providing a potential insight into involved biological networks. Those gene sets which were significantly correlated (multiple testing corrected $P < 0.05$) to genes increased in unfused sutures included genes with activating transcription factor 3 (ATF3) and lymphoid enhancer-binding factor 1 (LEF1) binding motifs within 2 kb of their transcription start sites, genes up-regulated by TGF β , genes up-regulated in haematopoietic stem cells, genes up-regulated in CD31 negative stromal stem cells which differentiate into bone cells, and genes down-regulated upon Cytomegalovirus (CMV) infection. This final gene set makes a connection between those genes down-regulated during suture fusion (i.e. up-regulated in unfused sutures) and genes down-regulated during infection. This integrated well with the gene sets which were significantly correlated with those genes increased during fusion; these included genes up-regulated in liver in graft versus host disease (GVHD; particularly genes associated with attraction and activation of donor T-cells), genes up-regulated in pulpal tissue from

Table 3: Selection of genes with fold change between unfused and fused sutures and between each unfused suture site.

Gene Name	Affymetrix ID	Description	Fold U-F	P-value U-F	Unfused Lambdoid	Unfused Coronal	Unfused Sagittal
Signalling							
ANGPTNL2	213004_at	angiopoietin-like 2	6.67	0.024	1.00	-1.45	-2.87
EGFR	201983_s_at	epidermal growth factor receptor	2.71	0.038	1.00	1.02	1.20
EPHA3	206071_s_at	EPH receptor A3	2.92	0.480	1.00	-2.06	-5.33
EPHA4	206114_at	EPH receptor A4	2.75	0.173	1.00	-1.19	-1.60
EPHB2	209589_s_at	EPH receptor B2	2.39	0.093	1.00	1.22	-1.12
FGFR2	208228_s_at	fibroblast growth factor receptor 2	3.36	0.294	1.00	-1.42	-3.72
FZD1	204451_at	frizzled homolog 1 (Drosophila)	2.13	0.326	1.00	-1.08	-2.41
IL11RA	204773_at	interleukin 11 receptor, alpha	5.90	0.003	1.00	1.49	1.32
TGFB2	209909_s_at	transforming growth factor, beta 2	3.76	0.096	1.00	-1.01	-2.08
FGFR1	222164_at	fibroblast growth factor receptor 1	1.23	0.480	1.00	-1.29	1.12
FGFR3	204379_s_at	fibroblast growth factor receptor 3	1.42	0.480	1.00	1.23	1.33
WNT5A	205990_s_at	wingless-type MMTV integration site family, member 5A	1.24	0.857	1.00	-1.03	-5.18
IL17R	205707_at	interleukin 17 receptor	-2.67	0.480	1.00	-1.25	3.35
OLFM4	212768_s_at	olfactomedin 4	-6.69	0.480	1.00	-2.15	6.77
WIF1	204712_at	WNT inhibitory factor 1	-3.19	0.480	1.00	-1.27	5.37
Structural							
COL2A1	213492_at	collagen, type II, alpha 1 (primary osteoarthritis)	7.21	0.390	1.00	-20.66	-3.84
COL3A1	215077_at	collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV)	6.32	0.072	1.00	1.24	-3.84
COL8A2	221900_at	collagen, type VIII, alpha 2	11.49	0.092	1.00	-1.23	-12.73
COL10A1	217428_s_at	collagen, type X, alpha 1 (Schmid metaphyseal chondrodysplasia)	14.65	0.407	1.00	-41.66	-17.28
FBLN1	202995_s_at	fibulin 1	12.88	0.025	1.00	-1.39	-1.92
FMOD	202709_at	fibromodulin	5.41	0.024	1.00	-1.09	-1.70
NELL2	203413_at	NEL-like 2 (chicken)	6.40	0.270	1.00	-4.21	-3.01
PRELP	204223_at	proline arginine-rich end leucine-rich repeat protein	10.65	0.009	1.00	1.20	-1.31
THBS2	203083_at	thrombospondin 2	6.62	0.069	1.00	-1.26	-2.47
COL1A2	202404_s_at	collagen, type I, alpha 2	1.05	0.480	1.00	-1.01	-1.06
CORO1A	209083_at	coronin, actin binding protein, 1A	-5.08	0.480	1.00	-1.05	11.73
Transcription							
BHLH3	221530_s_at	basic helix-loop-helix domain containing, class B, 3	5.95	0.093	1.00	-2.23	-4.76
CART1	206837_at	cartilage paired-class homeoprotein 1	2.06	0.533	1.00	16.21	2.38
HLF	204753_s_at	hepatic leukemia factor	3.54	0.210	1.00	-2.55	-6.81
JUN	201465_s_at	v-jun sarcoma virus 17 oncogene homolog (avian)	4.41	0.161	1.00	3.14	2.50
PITX2	207558_s_at	paired-like homeodomain transcription factor 2	3.45	0.279	1.00	1.63	-2.08
SIX2	206510_at	sine oculis homeobox homolog 2 (Drosophila)	2.72	0.136	1.00	-1.09	-1.70
FOS	209189_at	v-fos FBJ murine osteosarcoma viral oncogene homolog	1.40	0.745	1.00	14.96	4.13
FOSB	202768_at	FBJ murine osteosarcoma viral oncogene homolog B	1.83	0.695	1.00	54.37	20.22
FOXD1	206307_s_at	forkhead box D1	1.49	0.499	1.00	-4.71	-5.17
JUNB	201473_at	jun B proto-oncogene	1.28	0.703	1.00	3.61	2.95
MEOX2	206201_s_at	mesenchyme homeo box 2 (growth arrest-specific homeo box)	1.66	0.495	1.00	-3.99	-7.74
MSX2	210319_x_at	msh homeo box homolog 2 (Drosophila)	1.95	0.464	1.00	-1.14	-2.23
TWIST1	213943_at	twist homolog 1 (Saethre-Chotzen syndrome)	1.50	0.476	1.00	-1.20	-1.27
LHX6	219884_at	LIM homeobox 6	-1.80	0.050	1.00	-1.07	-1.03
SHOX2	210135_s_at	short stature homeobox 2	-3.44	0.480	1.00	-1.00	4.50
PAX5	221969_at	paired box gene 5 (B-cell lineage specific activator)	-4.20	0.480	1.00	-1.69	6.25
Catalytic Activity							
ROR1	205805_s_at	receptor tyrosine kinase-like orphan receptor 1	2.26	0.095	1.00	1.21	-1.14
HDHD1A	203974_at	haloacid dehalogenase-like hydrolase domain containing 1A	-1.78	0.018	1.00	1.05	1.05
ALOX5	204446_s_at	arachidonate 5-lipoxygenase	-5.55	0.480	1.00	-1.30	6.76
ANXA3	209369_at	annexin A3	-7.17	0.480	1.00	-1.30	6.76
Transporter							

Table 3: Selection of genes with fold change between unfused and fused sutures and between each unfused suture site. (Continued)

<i>CIQTNF3</i>	220988_s_at	CIq and tumor necrosis factor related protein 3	20.25	0.007	1.00	-1.52	-4.92
<i>RBP4</i>	219140_s_at	retinol binding protein 4 (plasma) KIAA1922	37.38	0.003	1.00	-1.35	-2.53
<i>APOC1</i>	204416_x_at	apolipoprotein C-I	-2.01	0.480	1.00	1.08	5.06
Binding							
<i>AGC1</i>	217161_x_at	aggrecan I (chondroitin sulfate proteoglycan I)	6.16	0.024	1.00	-1.25	-1.56
<i>CCND1</i>	208711_s_at	cyclin D1	2.13	0.449	1.00	1.38	2.24
<i>GPC3</i>	209220_at	glypican 3	7.13	0.050	1.00	1.26	-3.34
<i>HAPLN1</i>	205523_at	hyaluronan and proteoglycan link protein I	7.10	0.050	1.00	-1.96	-4.01
<i>INHBA</i>	210511_s_at	inhibin, beta A (activin A, activin AB alpha polypeptide)	6.94	0.006	1.00	-1.36	-3.03
<i>MFAP4</i>	212713_at	microfibrillar-associated protein 4	16.50	0.001	1.00	1.32	-1.86
<i>OGN</i>	218730_s_at	osteo glycin (osteoinductive factor, mimecan)	5.71	0.166	1.00	-1.41	-4.65
<i>POSTN</i>	214981_at	periostin (osteoblast specific factor 2)	5.87	0.101	1.00	-1.16	-1.60
<i>PTN</i>	209466_x_at	pleiotrophin (osteoblast specific factor I)	6.25	0.031	1.00	-1.14	-1.78
<i>S100A10</i>	200872_at	S100 calcium binding protein A10 (annexin II ligand)	2.01	0.096	1.00	-1.04	-1.60
<i>CCND3</i>	201700_at	cyclin D3	-2.60	0.480	1.00	-1.00	3.62
<i>FCN1</i>	205237_at	ficolin (collagen/fibrinogen domain containing) I	-5.45	0.480	1.00	-1.46	12.39
<i>FGR</i>	208438_s_at	Gardner-Rasheed feline sarcoma viral (v-fgr) oncogene homolog	-3.03	0.480	1.00	-1.13	5.75
<i>S100A12</i>	205863_at	S100 calcium binding protein A12 (calgranulin C)	-8.86	0.480	1.00	-2.63	15.19
Enzyme regulator							
<i>MMP14</i>	202827_s_at	matrix metalloproteinase 14 (membrane-inserted)	2.39	0.226	1.00	-1.22	-1.84
<i>TIMP3</i>	201149_s_at	tissue inhibitor of metalloproteinase 3	2.22	0.467	1.00	-1.71	-3.43
<i>CASP1</i>	211366_x_at	caspase 1, apoptosis-related cysteine protease	-2.73	0.165	1.00	-1.30	1.01
<i>MME</i>	203434_s_at	membrane metallo-endopeptidase (CD10)	-3.23	0.476	1.00	-2.35	-1.29
<i>MMP8</i>	207329_at	matrix metalloproteinase 8 (neutrophil collagenase)	-5.81	0.480	1.00	-2.36	1.69
<i>RASGRP2</i>	208206_s_at	RAS guanyl releasing protein 2 (calcium and DAG-regulated)	-2.60	0.480	1.00	1.02	3.02
Unknown Function							
<i>CYFIP2</i>	220999_s_at	cytoplasmic FMR1 interacting protein 2	-2.68	0.031	1.00	1.14	1.09

Gene expression fold change (U-F) is presented for unfused sutures (n = 8) compared to fused/fusing sutures (n = 6), with corresponding P-values, and for combined unfused coronal (n = 4) and unfused sagittal (n = 2) sutures compared to unfused lambdoid sutures (n = 2).

carious teeth, and genes up-regulated during retinoic acid induced promyelocytic differentiation. These observations are consistent with our previous GSEA observations in a microarray comparison between fused sutures tissue and de-differentiated explant cells, where we again found an increase in expression of immune response genes in fused suture tissues [29]. It is possible that these immune response genes reflect the formation of bone marrow within the fused bone matrix. In support of this, microscopy revealed a large accumulation of lymphocytes and other white blood cells within the calvarial bones (Additional file 7). A second explanation may be that premature fusion is functionally associated with an immune response to infection, either directly or indirectly, as it is known that various immunoregulatory cytokines influence bone homeostasis and that osteoblasts may facilitate immune responses by producing immunomodulatory molecules (reviewed in [30,31]).

Results were then analysed on a gene-based level. One of the families of genes which were significantly over-represented in unfused sutures was Eph/ephrin signalling molecules. These form a pathway recently invoked in causing

craniosynostosis [32,33]. Specifically, we found that three ephrin receptor genes had higher expression in unfused sutures (*EPHA3*, 2.9-fold; *EPHA4*, 2.8-fold; *EPHB2*, 2.4-fold). Multiple genes from several other gene families were also increased in unfused sutures (Table 3). These include small leucine-rich proteoglycans (SLRPs), a group of secreted proteins that are known to be involved in cartilage and bone formation through facilitating collagen fibril binding to the EMC [34,35] and regulating TGF β activity by sequestering TGF β in the ECM, thus preventing binding to cell surface receptors [36]. Such genes were, *proline arginine-rich end leucine-rich repeat protein* (*PRELP*, 10.7-fold), *osteo glycin* (*OGN*, 5.7-fold), *fibromodulin* (*FMOD*, 5.4-fold) and *decorin* (*DCN*, 2.0-fold).

A large over-representation of collagen genes was also observed. In particular, *collagen type II, III, VI, VIII, X, and XI* were all up-regulated in unfused sutures. Interestingly, Collagen type II and X are generally associated with cartilage formation, and would not be expected to be expressed during intramembranous ossification. However, a number of other cartilage-specific genes were also increased in unfused sutures (*AGC1*, 6.2-fold; *HAPLN1*,

Table 4: Gene Ontology analysis: unfused compared to fused sutures

Biological process increased in sutures:	Unfused	Fused	P-value
Cell adhesion	32		1.95×10^{-12}
Cell matrix adhesion	4		6.53×10^{-3}
Cell communication			
Cell surface receptor linked signal transduction	25		4.45×10^{-3}
Dopamine metabolism	2		6.63×10^{-4}
Phosphoinositide-mediated signalling		8	4.58×10^{-3}
Cell differentiation			
Heme biosynthesis		3	6.96×10^{-3}
Lymphocyte differentiation		6	1.27×10^{-3}
Cellular physiological process			
Anion transport	11		4.92×10^{-6}
Cell cycle		42	4.72×10^{-5}
Cell motility	11		2.70×10^{-4}
Extracellular matrix organisation and biosynthesis	4		1.11×10^{-3}
Microtubule based process		10	7.35×10^{-3}
Regulation of phosphorylation	3		5.30×10^{-3}
Morphogenesis	18		5.34×10^{-4}
Cellular morphogenesis	9		5.60×10^{-3}
Mesoderm formation	2		4.46×10^{-3}
Organ morphogenesis	8		7.46×10^{-3}
Organ development	23		3.45×10^{-7}
Cartilage condensation	2		5.89×10^{-3}
Eye development	3		4.98×10^{-4}
Muscle development	6		2.52×10^{-3}
Skeletal development	11		1.18×10^{-6}
Regulation of development			
Negative regulation of development	3		2.28×10^{-3}
Response to abiotic stimulus			
Response to chemical stimulus		20	7.31×10^{-3}
Response to reactive oxygen species		3	5.21×10^{-3}
Response to biotic stimulus		116	5.73×10^{-41}
Defence response to bacteria		11	5.2×10^{-9}
Defence response to fungi		3	1.65×10^{-3}
Response to virus		7	9.11×10^{-3}
Immune response		100	1.61×10^{-34}
Humoral immune response		22	1.57×10^{-8}
Inflammatory response		23	3.08×10^{-7}

Classification of gene ontologies (GO) significantly over-represented ($P < 0.01$) for genes at least 2 fold differentially expressed between unfused and fused sutures, listed under level 3 GO category headings. Not all level three GO categories listed were significantly over-represented and thus do not have a P-value

7.1-fold; *CART1*, 2.1-fold) suggesting that there may be a role for cartilage in calvarial suture morphogenesis. Additional secreted matrix proteins that were over-expressed in unfused suture tissue included *pleiotrophin* (*PTN*, also known as *osteoblast specific factor 1* (*OSF1*)) and *periostin* (*POSTN*, *osteoblast specific factor 2* (*OSF2*)). *PTN* has been identified in osteoblasts undergoing early stages of differentiation and is a potent regulator of osteoblast prolifera-

tion, recruitment, and differentiation [37]. *POSTN* is also expressed by early osteoblasts and is a target of *Twist1* transcriptional regulation in mice [38]. A number of proteases and protease inhibitors were also differentially expressed between unfused and fused suture tissue including, *MMP2* (2.0-fold), *MMP14* (2.4-fold), *MMP8* (5.8-fold), *MME* (-3.23-fold), *SERPINA1* (-2.7-fold), *SERPINB1* (-3.6-fold) and *TIMP3* (2.3-fold) (Additional file 5).

Numerous genes involved in Wnt signalling were also identified; *glypican 3* (*GPC3*, 7.1-fold) and *frizzled 1* (*FZD1*, 2.1-fold) were increased in unfused sutures, while *WNT inhibitory factor 1* (*WIF1*, 3.2-fold) was increased in fused sutures. Previously, we have identified an up-regulation of *WIF1* in human fused suture tissue when comparing *in vivo* expression to expression of de-differentiated explant cells [29]. These results are consistent with the recent observations that activation of canonical Wnt signalling is important in osteoblast expansion and differentiation [39], and that antagonist of Wnt signalling are essential to initiate terminal osteoblasts differentiation [40].

A number of the genes differentially expressed between fused, fusing, and unfused sutures which had a large significant ($P < 0.05$) fold change (Table 2) had not been previously identified to be expressed in human calvaria. Of particular interest were *retinol-binding protein 4* (*RBP4*, 37.4-fold, $P = 0.003$), *C1q and tumour necrosis factor related protein 3* (*C1QTNF3*, 20.3-fold, $P = 0.007$), *microfibrillar-associated protein 4* (*MFAP4*, 16.5-fold, $P = 0.001$), *PRELP* (10.7-fold, $P = 0.009$), *GPC3* (7.1-fold, $P = 0.05$), *tenascin N* (*TNN*, 6.7-fold, $P = 0.038$), *pleiotrophin* (*PTN*, 6.3-fold, $P = 0.031$) and *interleukin 11 receptor alpha* (*IL11RA*, 5.9-fold, $P = 0.003$). The significant expression of all these genes for the combined suture comparison suggests that they are likely to be key regulators of morphogenesis in all sutures.

Unfused sagittal sutures have a lower expression of the 'unfused' class of genes

To analyse the effect of suture type on gene expression we used two methods of analysis to compare global expression data between unfused coronal, sagittal, and lambdoid sutures. We then compared results from the two methods to identify the most robust set of differentially expressed genes. For the 3-way analysis we used a linear modelling approach to jointly perform three pair-wise comparisons: coronal vs sagittal, lambdoid vs sagittal, and lambdoid vs coronal and we then identified where these groups overlapped (Fig. 3). This pooled approach provides greater sensitivity and statistical power over multiple direct comparisons. Unfused sagittal sutures were found to have 340 probe sets differentially expressed when com-

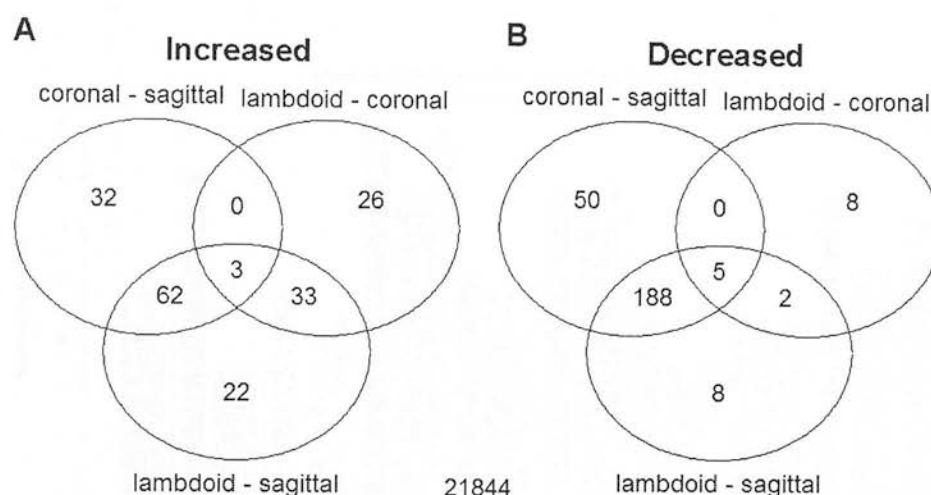


Figure 3
Venn diagram of 3-way unfused suture comparison results. Global expression differences were compared for 3 pair-wise comparisons (coronal vs sagittal, lambdoid vs coronal, lambdoid vs sagittal). The sagittal suture showed the greatest difference in gene expression to the other two sutures, followed by the lambdoid suture. **A)** Increased genes. 62 genes had similar increased expression in coronal and lambdoid sutures compared to sagittal sutures and 33 genes had increased expression in lambdoid sutures and similar expression in coronal and sagittal sutures. **B)** Decreased genes. 188 genes had similar decreased expression in unfused coronal and lambdoid sutures compared to unfused sagittal sutures and 2 genes had decreased expression in lambdoid sutures and similar expression in coronal and sagittal sutures.

pared to coronal sutures and 323 when compared to lambdoid sutures, while the coronal and lambdoid sutures only had 77 probe sets differentially expressed between each other. Furthermore, 250 of those probe sets differentially expressed in unfused sagittal were not differentially expressed between the coronal and lambdoid sutures. This supported the higher correlation of gene expression of differentially expressed genes between unfused coronal and lambdoid sutures seen in Figure 2. Of these 250 probe sets, 62 were increased in unfused coronal and lambdoid sutures and 188 were decreased, compared to the sagittal sutures (Fig. 3). The 3-way analysis also showed that 35 probe sets were uniquely expressed in unfused lambdoid sutures compared to coronal and sagittal sutures (33 increased and 2 decreased).

To control for patient-specific effects we performed a set of separate pair-wise comparisons using a matched pairs design. In this case we first restricted analysis to patients with a sample from each of the sutures of interest and then performed analysis on the within patient differences. A list of significant genes with $P < 0.01$ was produced from each pair-wise comparison (Additional files 8, 9, 10).

The combination of the 3-way and pair-wise comparisons identified 100 probe sets significantly ($P < 0.01$) differentially expressed in unfused sagittal sutures compared to unfused coronal and lambdoid sutures. Amongst the top ten genes, seven had higher expression in unfused sagittal sutures and two had lower expression compared to coro-

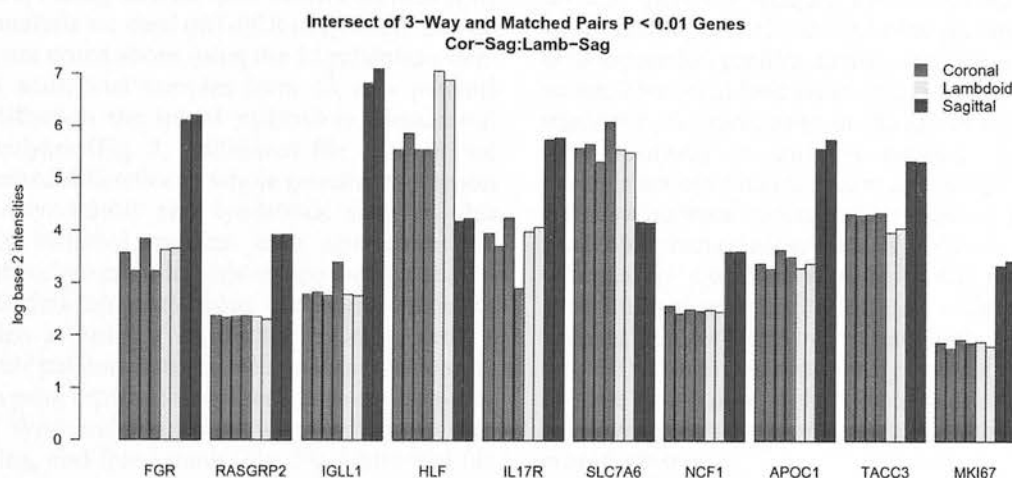
nal and lambdoid sutures (Fig. 4). Outside the top ten, genes with significantly decreased expression in unfused sagittal sutures were *FGFR2*, *MSX2*, *GPC3*, and *WNT5A*, while the Wnt inhibitor *WIF1* had increased expression. The observed trend was that unfused sagittal sutures have a lower expression of those genes typically associated with an unfused suture state and a higher expression of genes associated with suture fusion (Table 3).

Unfused coronal and sagittal sutures have differential expression of transcription factors compared to lambdoid sutures

Analysis of the unfused-suture comparison data with respect to genes differentially expressed by unfused lambdoid sutures identified a large number of transcription factors. Those down-regulated with respect to coronal and sagittal sutures included *FOS*, *FOSB*, *JUN*, *JUNB*, and *CART1*; all having a tendency for greater expression in the coronal suture (Table 3). Those genes with higher expression in unfused lambdoid sutures included transcription factors *FOXD1*, *MEOX2*, *HLF* and *BHLH3*.

Microarray results validated by real-time quantitative RT-PCR and Western blot

Results from the microarray analysis were validated by qRT-PCR for 11 of the most highly expressed and significantly differentially expressed genes: eight genes increased in unfused suture tissue, *RBP4*, *C1QTNF3*, *PRELP*, *GPC3*, *PTN*, *FMOD*, *COL3A1*, and *COL8A2*; and 3 genes increased in fusing/fused suture tissue, *WIF1*, *ANXA3*, and

**Figure 4**

Top ten genes differentially expressed between unfused coronal, lambdoid and sagittal sutures. Genes commonly identified to be differentially expressed ($P < 0.01$) in unfused coronal and lambdoid sutures compared to unfused sagittal sutures, using two methods of microarray data analysis: 3-way and matched-pairs. Eight genes (*FGR*, *RASGRP2*, *IGLL1*, *IL17R*, *NCF1*, *APOC1*, *TACC3*, and *MKI67*) have higher expression in unfused sagittal sutures and two (*HLF* and *SLC7A6*) have lower expression compared to unfused coronal and lambdoid sutures. *HLF* shows a gradient of expression, highest in lambdoid, then coronal, then sagittal sutures, whereas the other genes show similar expression in coronal and lambdoid sutures. For each gene listed, sutures are in patient order: coronal (#36, #46, #50, #58), lambdoid (#46, #58), sagittal (#36, #58).

CYFIP2. The Affymetrix probe set for *C1QTNF3* targeted two transcripts and therefore two transcript-specific primers were designed. Gene expression was analysed using 10 of the same RNA samples which underwent microarray analysis (Table 1). A linear correlation was calculated for each transcript for the comparison of expression values obtained by qRT-PCR and microarray analysis (Table 5). An average correlation of 90% was observed for all genes analysed, validating the microarray results. Three

primer sets had a correlation coefficient smaller than 75%; however, two of these primer sets did not amplify all isoforms detected by their corresponding Affymetrix probe set. The third primer set was designed to detect the long isoform of *C1QTNF3*. While this had a correlation of 74%, the short isoform *C1QTNF3* primers had 99% correlation, suggesting that it is the short isoform of *C1QTNF3* that is differentially expressed.

Table 5: Linear correlation between microarray and qRT-PCR data.

Gene	Probe Set	Slope	Correlation
<i>ANXA3</i>	209369_at	0.881	0.978
<i>WIF1</i>	204712_at	0.850	0.982
<i>CYFIP2</i>	220999_s_at	0.785	0.748
<i>PTN</i>	211737_x_at	1.063	0.905
<i>PRELP</i>	204223_at	0.755	0.938
<i>FMOD</i>	202709_at	0.867	0.851
<i>C1QTNF3</i> long isoform	220988_s_at	1.774	0.738
<i>C1QTNF3</i> short isoform	220988_s_at	0.806	0.990
<i>RBP4</i>	219140_s_at	1.100	0.968
<i>GCP3</i>	209220_at	1.036	0.929
<i>COL8A2</i>	221900_at	1.261	0.854
<i>COL3A1</i>	215077_at	0.767	0.709
Average Correlation			0.896

The average correlation for all probe sets is approximately 90%, not including the correlation of the long isoform of *C1QTNF3*. The short isoform of *C1QTNF3* had 99% correlation, indicating that the short and not the long isoform of *C1QTNF3* is differentially expressed between unfused and fused sutures.

To further examine the differential expression of genes between fused, fusing and unfused sutures identified by microarray analysis we used qRT-PCR to quantify expression of all genes noted above using the 10 validation samples and 25 additional samples from 13 new patients (Table 1). Although the initial microarray hierarchical clustering analyses (Fig. 2, Additional file 4) indicated there was limited difference in whole genome expression between non-syndromic and syndromic samples, this analysis only included samples from one syndromic patient. We therefore extended this comparison by analysing qRT-PCR data obtained from the larger cohort of patients which included 7 syndromic patients, and 11 non-syndromic patients in total. No significant difference ($P < 0.05$) in gene expression was seen between non-syndromic and syndromic samples, when separated into unfused, fusing, and fused states (Fig. 5A, Additional file 11). This result clearly demonstrates that samples of different aetiologies can be combined to investigate the general mechanisms of craniosynostosis.

As the unfused suture samples which underwent microarray and qRT-PCR analyses were obtained from patients with craniosynostosis, there is the possibility that their gene expression profiles do not truly represent an unfused suture from an individual without craniosynostosis. Consequently, the expression of the above mentioned genes were analysed in unfused coronal, lambdoid, and metopic sutures obtained from similar age-matched individuals who were undergoing transcranial surgery for reasons other than craniosynostosis (Table 1). No significant difference ($P < 0.05$) in gene expression was observed for these unfused non-craniosynostosis sutures, compared to unfused sutures from individuals with craniosynostosis (Fig. 5B). This provides proof of principle that the analysis of gene expression profiles from unfused sutures from craniosynostosis patients is useful in the study of suture morphogenesis.

qRT-PCR data was then compared between unfused, fusing, and fused samples from each suture site, combining

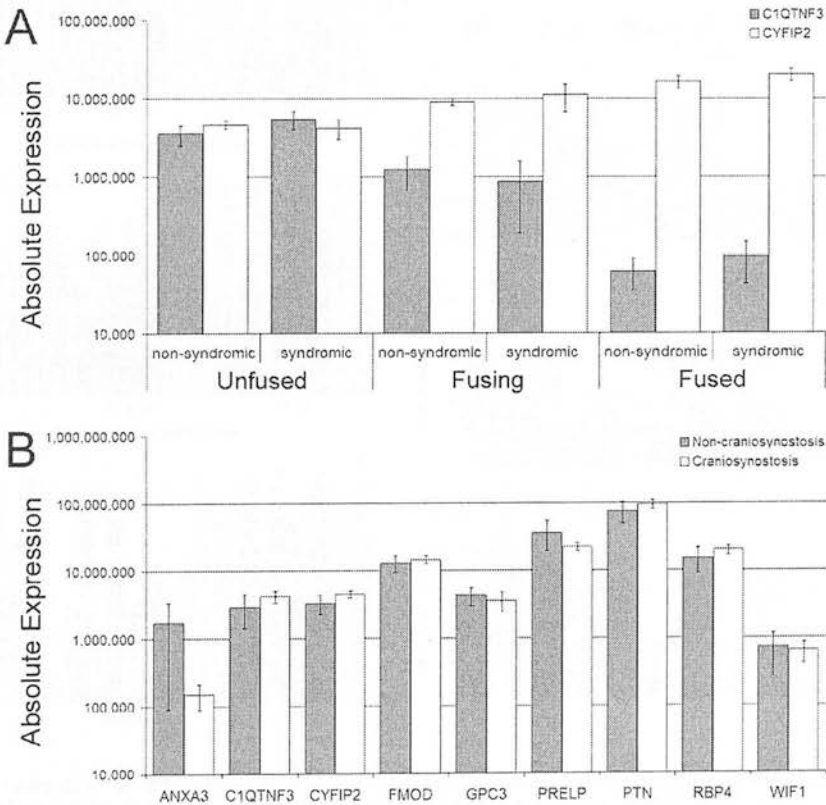


Figure 5
qRT-PCR expression data comparing syndromic, non-syndromic and non-craniosynostosis samples. **A)** No significant difference in expression exists between suture samples from syndromic and non-syndromic craniosynostosis patients. *C1QTNF3* is decreased during fusion and *CYFIP2* is increased during fusion, irrespective of aetiology. Samples from coronal, sagittal, lambdoid, and metopic sutures were combined for analysis. Samples numbers (left to right) are n = 8, 4, 5, 4, 7, 7. **B)** Unfused sutures from craniosynostosis patients (n = 12) and individuals without craniosynostosis (n = 6) show no significant difference ($P < 0.05$) in expression for all genes analysed. Absolute expression values represent molecules per ng cDNA. Mean expression \pm SEM is shown.

non-syndromic and syndromic patients together. Differential expression profiles were observed for all 11 genes analysed, although the level of differential expression varied between suture sites (Fig. 6A, Additional file 12). The greatest difference in expression was observed for coronal sutures, followed closely by lambdoid sutures, while metopic sutures had, in general, the smallest changes in expression between unfused and fused sutures. This later observation is likely due to the finding that unfused metopic sutures generally had a lower level of expression of genes increased in unfused sutures (eg. *C1QTNF3*, *FMOD* and *PTN*) and a higher expression of genes which were increased in fused sutures (eg. *WIF1* and *ANXA3*).

Unfused sagittal sutures also showed higher expression than unfused coronal and lambdoid sutures for those genes increased in fused sutures (*ANXA3* and *WIF1*) (Fig. 6A). These combined patient results confirmed the suture-specific analyses outlined earlier (Table 3, Fig. 2). The qRT-PCR validation experiment also demonstrated a variable gradient of expression between unfused, fusing, and fused samples for all genes analysed (Fig. 5A and Fig. 6A). However, this gradient of expression is best analysed using samples isolated from the same sutures from the same patient that are undergoing various stages of fusion. Figure 7 shows the qRT-PCR data from 5 samples isolated from one Apert syndrome patient (#90). Two fusing coro-

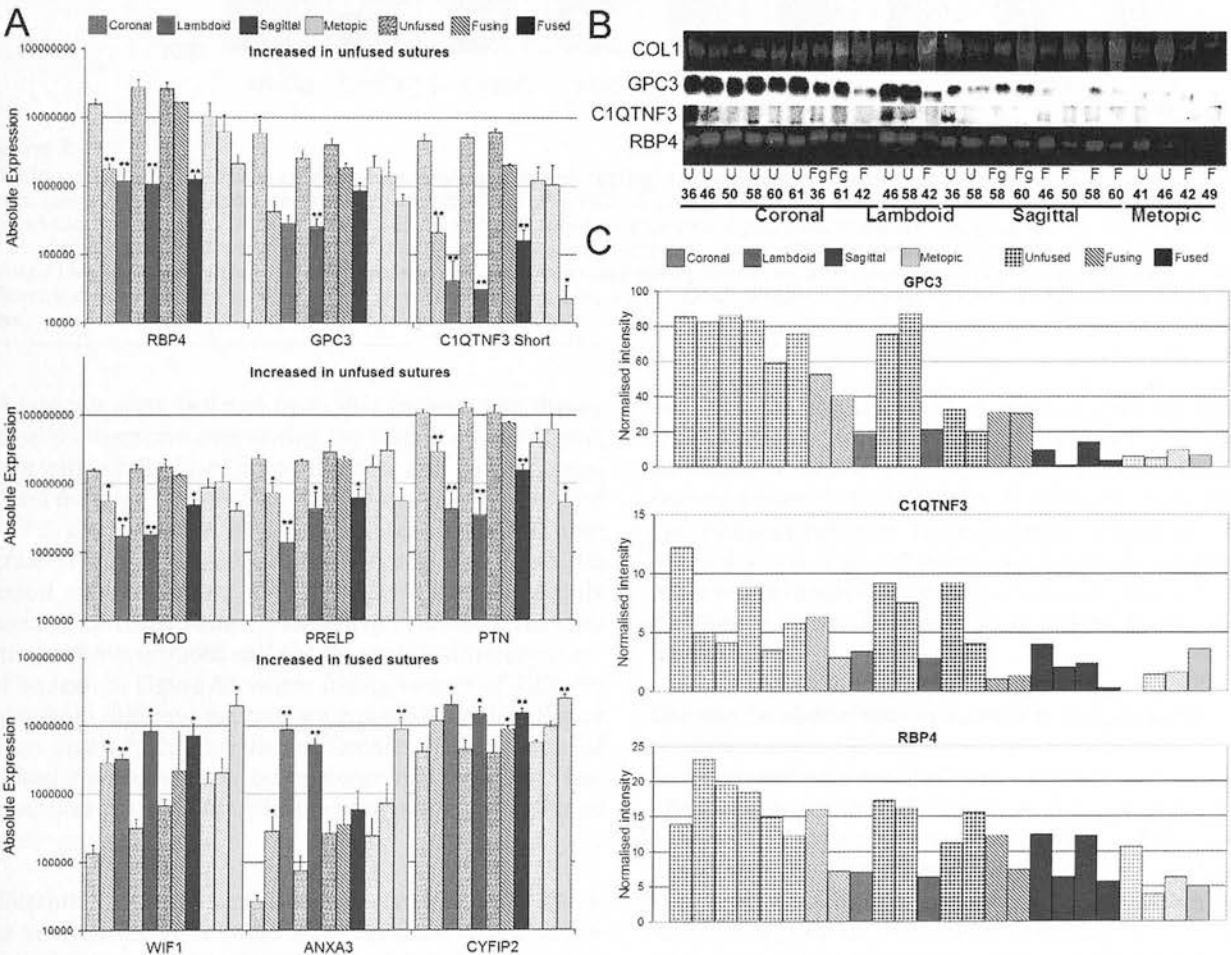


Figure 6
mRNA and protein validation of differential expression identified by microarray analysis. A) Real-time qRT-PCR analysis of six genes with increased expression in unfused sutures (*RBP4*, *GPC3*, *C1QTNF3* short isoform, *FMOD*, *PRELP*, and *PTN*) and three genes with increased expression in fused sutures (*WIF1*, *ANXA3*, and *CYFIP2*) for unfused, fusing and fused suture tissue isolated from sagittal, coronal, lambdoid and metopic sutures. Significant differential expression ($P < 0.05$, *; $P < 0.01$, **) was analysed for fusing and fused sutures compared to unfused sutures. Mean expression + SEM is shown; $n = 3$ for all comparisons, except fused sagittal ($n = 5$), fused metopic ($n = 4$), and fused lambdoid ($n = 2$). Absolute expression values represent molecules per ng cDNA. **B)** Western blot analysis of individual protein samples in the order seen in (C), for collagen type I (COL1), GPC3, C1QTNF3 and RBP4. **C)** Densitometry analysis of western blots normalised to COL1 expression.

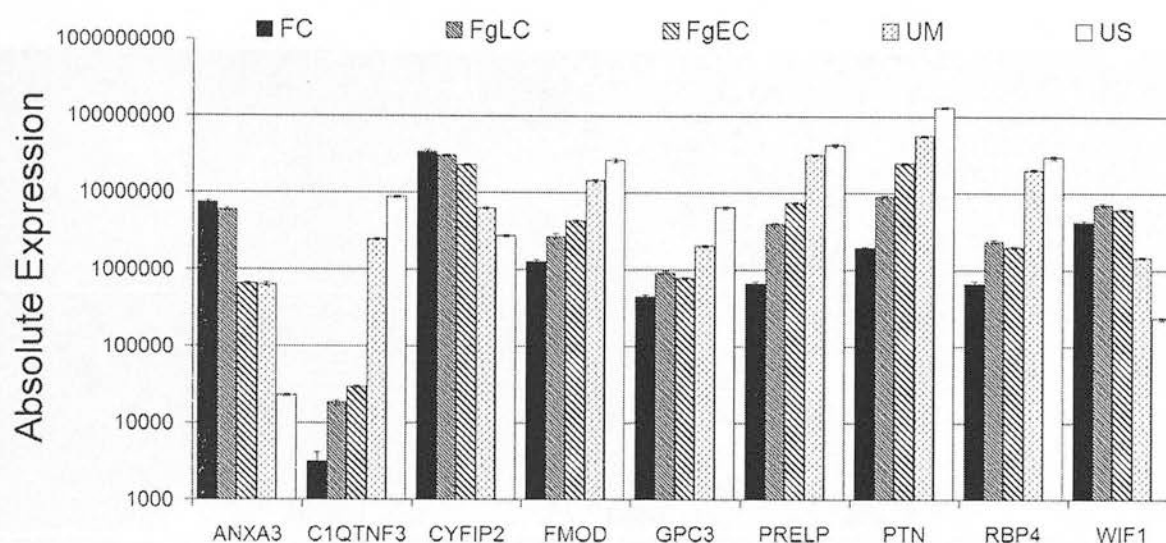


Figure 7
Within patient comparison of gene expression in fused, fusing, and unfused sutures. Gene expression was analysed in five suture samples from an Apert syndrome patient (#90) by qRT-PCR. A gradient of expression is seen for all genes from unfused sagittal (US), to unfused metopic (UM), early fusing coronal (FgEC), late fusing coronal (FgLC), and fused coronal (FC) sutures. *ANXA3* and *CYFIP2* are increased highest in fused sutures, *WIF1* in fusing sutures and all other genes have highest expression in unfused sagittal sutures. Unfused Metopic sutures show higher expression of genes increased during fusion and a lower expression of genes increased in other unfused sutures. Absolute expression values represent molecules per ng cDNA. Error bars represent SEM for triplicate technical replicates.

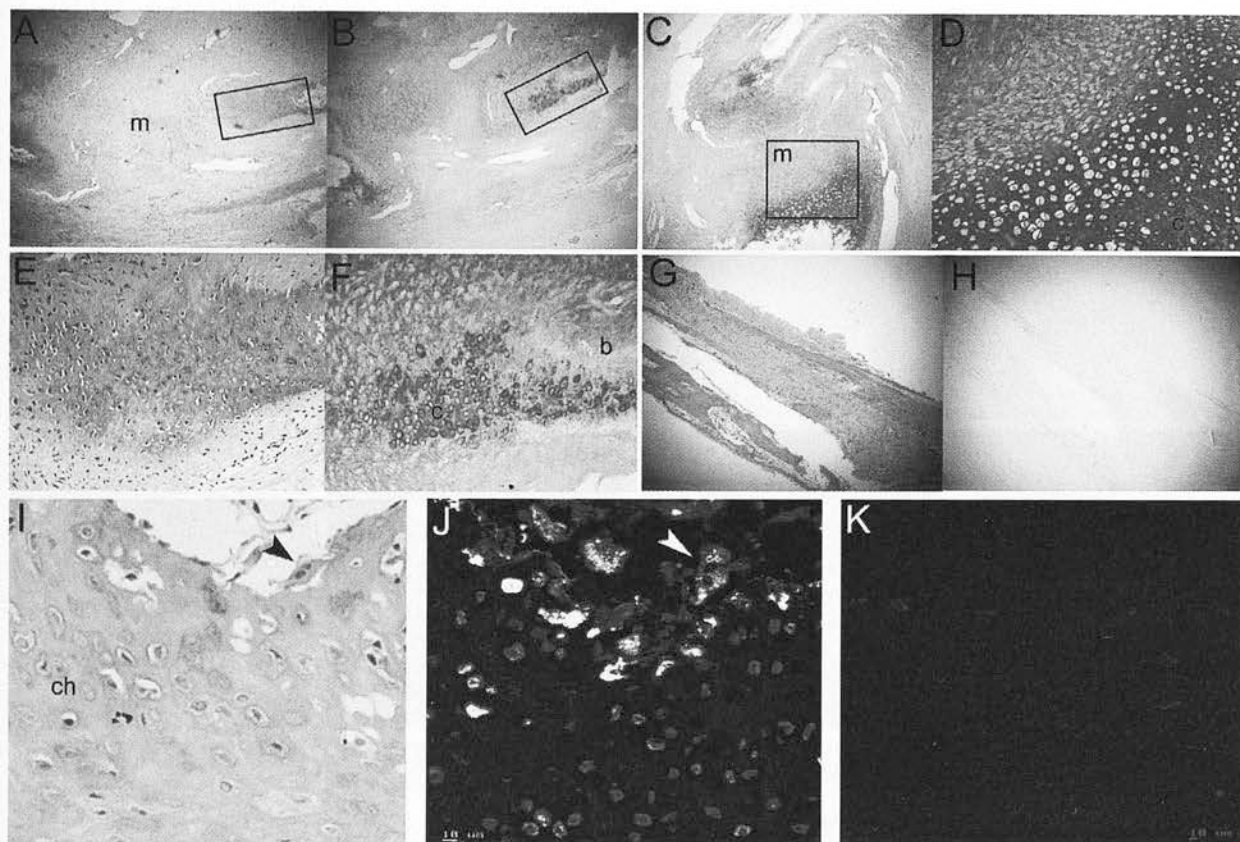
nal samples were isolated from this patient, one during the early stages and one during the later stages of fusion, along with a fully fused coronal suture and unfused sagittal and metopic sutures. The data shows that *ANXA3* and *CYFIP2* are increased in fusing sutures, but are further increased in fully fused sutures, whereas *WIF1* has the greatest expression in fusing sutures and is slightly decreased once the suture is fully fused, however this level remains above unfused sutures. This subtle difference cannot be seen in Figure 6A where fusing sutures of different stages from different patients are grouped together. Figure 7 also again highlights the difference in expression of unfused metopic sutures, being closer in expression to fusing sutures than other unfused sutures for a number of genes.

Differential protein expression was assessed by Western blot analysis for three genes with increased expression in unfused sutures, *RBP4*, *C1QTNF3* and *GPC3* (Fig. 6B and 6C). Microarray results indicated that collagen type I alpha 2 was the most abundant transcript and was not differentially expressed between unfused and fused suture tissues (1.05 fold, Table 3). Protein expression was therefore normalised to *COL1* for comparative quantification. All three proteins were differentially expressed in a similar pattern to that observed for the RNA expression data, with a decreasing gradient of expression observed for unfused, fusing, and then fused samples. Again, lower expression of

each protein was observed in unfused metopic sutures. Higher protein expression was observed in two fused sagittal sutures (#46 and #58) compared to two other fused sagittal sutures (#50 and #60). This may be explained in part by tissue structure. During sample preparation it was noted that #46 and #58 sutures were very thin, flat bones more representative of developed calvaria, while #50 and #60 were more archetypal, having enlarged fused-suture ridges [5].

Unfused Lambdoid sutures express cartilage-specific markers

Increased expression of *COL10A1* and *COL2A1* was identified in unfused lambdoid sutures and one (#58) of the two unfused sagittal sutures compared to all other samples (Table 3). The unfused sagittal suture from patient #58 was taken from the extreme posterior portion of the suture very close to the lambdoid suture (Fig. 1A). In all other gene expression analyses this sample grouped with the other unfused sagittal suture, verifying its correct classification as sagittal suture tissue. The expression of cartilage-specific collagens suggested, contrary to conventional thinking, that cartilage may play a role in human suture morphogenesis. Histological analysis of fused and unfused lambdoid, coronal, and sagittal sutures from additional patients to those used for microarray analyses (Table 1), identified cartilage only in unfused lambdoid sutures (Fig. 8). Cartilage was found at the tips of the oste-

**Figure 8**

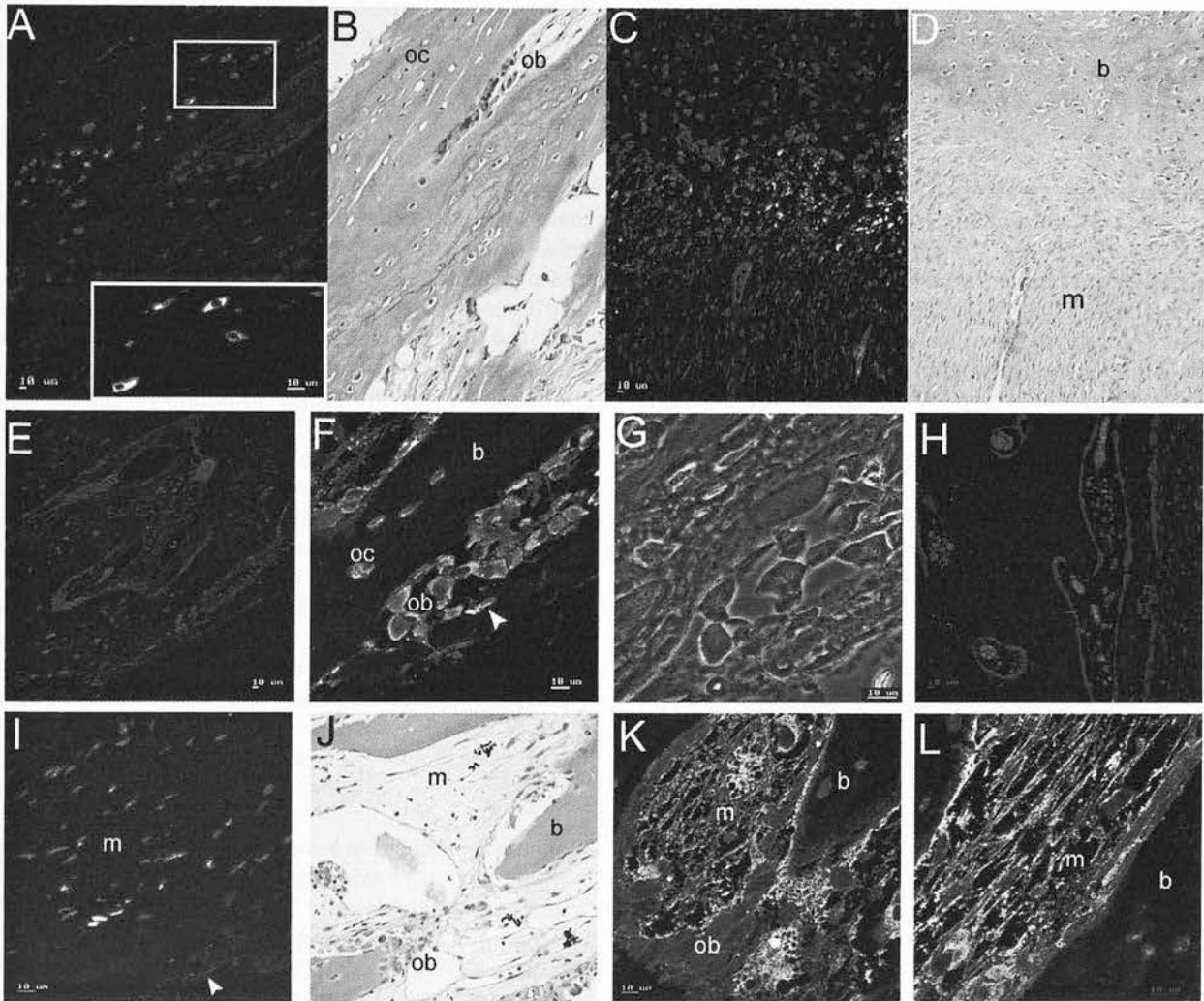
Cartilage localisation in unfused sutures. **A-B)** Serial H&E and Alcian blue stain of right unfused lambdoid suture (#83) showing cartilage (boxed regions expanded in panels E and F, respectively) on either side of suture mesenchyme (m). **C)** Alcian blue stain of left unfused lambdoid suture (#83) showing cartilage fronts (box) surrounding suture mesenchyme. **D)** Cartilage front from panel C (box) showing proliferating (stacked cells) and hypertrophic (cells with enlarged lacunae) chondrocytes in a cartilage (c) matrix. **E-F)** Enlarged views of boxed regions in A and B, respectively showing H&E (E) and Alcian blue (F) stain of right unfused lambdoid suture (#83) and highlighting cartilage interspersed with calcified bone (b, dark pink). Hematoxylin stains calcified matrix darker. **G-H)** Serial H&E (G) and Alcian blue (H) staining of an unfused coronal suture (#83) showing no staining of cartilage in (H). **I-J)** H&E (I) and confocal immunofluorescence for Collagen type X (J) detected weak localisation (orange) in hypertrophic chondrocytes (ch), with intense (yellow) punctate localisation in osteoclasts (multi-nucleated cells, arrowhead) adjacent to the cartilage matrix of unfused lambdoid sutures (#83). **K)** Collagen type X protein was not detected in osteogenic fronts of unfused coronal sutures (#83). Magnification: A-C, G-H: X3.2; D-F: X12.5; Scale: I-K: 10 μ m.

ogenic fronts which protruded into the suture mesenchyme. This cartilaginous region was composed of what histologically appeared to be a region of proliferating and hypertrophic chondrocytes, and was seen to protrude into the osteoblastic region where calcification was occurring to form calvarial bone (Fig. 8A-8F). The spatial expression of COLX protein, which is a specific marker of hypertrophic chondrocytes [41], was investigated by confocal immunofluorescence (Table 1). COLX was found to be highly abundant in osteoclasts, which were localised to the edges of the cartilage matrix adjacent to the bone matrix. COLX was also expressed, but more weakly, by chondrocytes in the cartilage matrix (Fig. 8I, J). COLX

expression was not identified in any other suture type (Fig. 8K).

Different spatial localisation of RBP4 and GPC3 in unfused sutures

Tissue localisation of RBP4 and GPC3 was investigated using confocal microscopy in fused and unfused tissue from coronal, sagittal, and lambdoid sutures (Fig. 9). In all unfused sutures, RBP4 was located in the cytoplasm, the most intense staining being in osteocytes in the outermost region of bone overlying the suture region on the ectocranial but not the endocranial surface (Fig. 9A, B, E). RBP4 expression was also observed in osteoblasts at the osteogenic fronts (Fig. 9C, D), those invaginating the oste-

**Figure 9**

Localisation of RBP4 and GPC3 in suture tissue. **A-B)** Immunofluorescence and H&E stain showing intense localisation (yellow) of RBP4 in the cytoplasm of osteocytes (oc) in ectocranial surface bone (unfused coronal suture, #83). **C-D)** Serial immunofluorescence (C) and H&E sections (D) showing RBP4 located in cells in the region between calcified tissue (b) and mesenchyme (m) (unfused left lambdoid suture, #83). **E)** RBP4 was not detected on the endocranial surface of unfused sutures (coronal, #83). **F)** RBP4 was localised to the cytoplasm of osteoblasts (ob) lining the developing bone, those being trapped in the osteoid (arrow head), and osteocytes (unfused coronal suture, #83). **G)** Corresponding phase contrast image to the central region in (F). **H)** RBP4 was not detected in fused sutures. Red blood cells had weak autofluorescence (sagittal, #5). **I-L)** GPC3 immunofluorescence (I) and H&E (J) detected protein in mesenchymal cells close to the tissue surface (arrow head) in the mid-suture region (unfused sagittal suture, #5). Membrane staining was observed for the cytoplasmic extensions of mesenchymal cells adjacent to calcified bone (K-L, unfused coronal suture, #83). **J)** H&E of section deep to (K) showing calcified bone protruding into intervening mesenchyme with osteoblasts lining the bone. Scale: 10 μm

oid, and by osteocytes at the bone margin (Fig. 9F, G), but not in osteocytes distal from the suture region. In the lambdoid sutures, RBP4 expression was also high in osteoblastic cells lining the cartilage fronts. No specific staining was observed in fused sutures (Fig. 9H).

Glypican 3 was most highly expressed in suture mesenchyme, particularly adjacent to the osteogenic fronts.

There was distinct cell surface staining of mesenchymal cells, clearly showing the delicate branching of their cytoplasmic extensions, forming an interlacing network throughout the suture space between bone fronts (Fig. 9J-L). Osteoblasts lining osteogenic fronts and those recently invaginated also had membranous staining although considerably weaker. There was also staining of mesenchymal cells close to the tissue surface in the mid-

sutural region (Fig. 91). The higher expression in mesenchymal cells correlates with the higher mRNA and protein expression in unfused compared to fusing and fused sutures (Fig. 6).

Discussion

Here, we have identified novel genes and attempted to gain a broader understanding of the various molecular pathways controlling suture morphogenesis in humans postnatally by analysing global gene expression differences between unfused sutures and prematurely fusing/fused sutures from patients with craniosynostosis. This is the first study in which microarray analysis has been applied to investigate differential gene expression in fused, fusing, and unfused human sutures. We identified differentially expressed genes in pathways that have been a major focus of study in craniosynostosis, including FGF, TGF β and EGF signalling pathways. In addition, we identified genes from the Eph/ephrin pathway that has recently been linked with craniosynostosis [32,33] and the Wnt pathway that is involved osteoblast differentiation [42] and transducing FGFR signals [43]. A number of novel genes which may have important roles in suture biology and which have not been previously linked with craniosynostosis have also been identified, specifically *RBP4*, *GPC3*, and *C1QTNF3*. All three are abundantly expressed in unfused sutures and are significantly down-regulated in prematurely fused sutures.

A role for retinol binding protein 4 in regulating retinoic acid induced osteoblast differentiation

Retinol binding protein 4 (*RBP4*) expression was decreased 37-fold in prematurely fused/fusing sutures compared to unfused sutures by microarray analysis. *RBP4* is a secretable plasma protein that classically transports retinol from liver stores to tissues where it undergoes catalytic conversion into retinoic acid (RA) [44]. Extrahepatic *RBP4* expression occurs in adipose, kidney, cartilage, and brain tissue and during embryonic development of the orofacial region [45-48]. It has been speculated that tissue-specific expression of *RBP4* is linked to the binding of retinol from plasma in the immediate vicinity of, and for specific use by, its tissue of origin [49,50]. Our finding of *RBP4* in osteocytes on the ectocranial surface of bone directly overlying the suture mesenchyme and by cells lining the osteogenic fronts, and its dramatic downregulation in suture fusion now implicates a role for it in suture morphogenesis and craniosynostosis. At teratogenic levels, the metabolite of retinol, RA, causes craniosynostosis and other developmental anomalies [4,51,52]. At physiologic levels, however, RA represses growth of preosteoblastic cells and enhances the expression of *alkaline phosphatase*, *osteonec-tin*, *osteopontin*, *collagen type I* and the osteoblast specific transcription factor *RUNX2* [53,54]. RA also stimulates *BMP2* expression and cooperates to induce osteoblast dif-

ferentiation [55]. Furthermore, primary rat calvarial osteoblasts treated with RA have increased *osteopontin* expression and switch from predominately expressing *FGFR2* to *FGFR1*, representing a switch from active proliferation to osteoblast differentiation [56]. At physiologic levels, a function of this metabolite of retinol is therefore to suppress preosteoblastic proliferation and activate differentiation. This role of RA correlates with the localisation, reported here, of *RBP4*, the specific transporter of its precursor, in cells lining the ectocranial surface and the osteogenic fronts of unfused sutures and in those recently invaginated into the osteoid. *RBP4* may therefore represent a primary regulator of osteogenesis in calvarial sutures by mediating the availability of retinol and its subsequent conversion to RA. In support of this, *RBP4* knockout mice develop cranial malformations [57]. Taken together with our data on *RBP4* expression during suture fusion and epidemiological evidence linking excess RA with craniosynostosis and other developmental anomalies [4,51,52,58] we speculate that perturbations in the *RBP4*-retinol-RA axis may contribute to the occurrence of craniosynostosis.

A role for glypican 3 in maintaining suture patency

Glypican 3 (*GPC3*) is a cell surface heparan sulfate proteoglycan which binds to the extracellular surface of cells via a GPI (glycosyl-phosphatidyl-inisitol) anchor and is thought to facilitate interaction between various ligands and receptors. Loss-of-function mutations in *GPC3* cause Simpson-Golabi Behmel syndrome (MIM 312870), an overgrowth syndrome with multiple skeletal abnormalities (large protruding jaw, widened nasal bridge, upturned nasal tip, and broad, short hands and fingers) that is associated with increased cell proliferation [59]. Our study shows that *GPC3* expression is decreased 7-fold in prematurely fused/fusing sutures. *GPC3* interacts with FGF2, WNT5a, and BMP-4 and -7 which are ligands of FGF receptors, WNT receptors (FZDs), and BMP receptors, respectively [60-62], all of which have been variously implicated in regulating osteoblast function. Molecular studies show that loss of *GPC3* enhances the limb patterning defect of *BMP4* heterozygous mice [62] and that *GPC3* can bind FGF2 and suppress FGF2-induced cell proliferation [61]. *GPC3* is also able to regulate the Wnt signalling pathway. *GPC3* knockout mice exhibit an inhibition of the non-canonical Wnt/JNK signalling pathway, and activation of the canonical Wnt/ β -catenin signalling pathway [63]. Canonical Wnt/ β -Catenin signalling promotes osteoblast differentiation and bone accrual and inhibits osteoblast apoptosis (reviewed in [39]). We speculate that *GPC3* controls cell growth within suture mesenchyme by regulating the bioavailability of FGFs, BMPs, and Wnts and might therefore acts as a gate-keeper of cell responsiveness in the suture.

A role for *C1QTNF3* in suture morphogenesis

C1QTNF3 (C1q tumor necrosis factor related protein 3), also known as CTRP3/cartducin, and CORS26, is a growth factor that hitherto had not been linked to suture morphogenesis. Our microarray study showed that *C1QTNF3* expression is decreased 20-fold in prematurely fused/fusing sutures and qRT-PCR analysis determined that it is the short isoform of *C1QTNF3* that is differentially expressed (Table 5, Fig. 6A). *C1QTNF3* regulates proliferation of chondrocytes and their progenitor cells during both post-natal and embryonic development and has been shown to be up-regulated during BMP2 and insulin induction of chondrocyte differentiation [64]. *C1QTNF3* has also been identified as promoting proliferation and migration of mouse endothelial MSS1 cells [65]. With the discovery of *C1QTNF3* expression in unfused and fusing tissue of all sutures (Fig. 6), we predict a novel role for this growth factor in the regulation of osteoprogenitor proliferation and differentiation at the osteogenic fronts. Given our finding of cartilage-specific markers associated with posterior skull sutures we speculate that *C1QTNF3* might also be involved in chondrogenesis in addition to osteogenesis in these sutures.

The identification of cartilage in posterior sutures

Of particular interest was the identification in our microarray experiment of increased expression of genes coding for cartilage-specific *collagens types II and X* in the two unfused lambdoid and the unfused sagittal sutures from the posterior of the skull. The involvement of cartilage in unfused lambdoid sutures was confirmed histologically through the observation within the region of the osteogenic fronts of a cartilaginous matrix which protruded into the suture mesenchyme and adjacent calcified bone matrix (Fig. 8). In addition, confocal microscopy localised collagen type X to chondrocytes in the cartilage matrix and in osteoclasts adjacent to cartilage (Fig. 8I, J). Identification of what is termed 'secondary cartilage' has been previously noted in several human calvarial sutures, with a high incidence in normal lambdoid sutures [66,67]. It has been proposed that this secondary cartilage may develop in response to the higher mechanical forces applied to the posterior region of the skull during growth as it provides a matrix more tolerant to compression [5]. Cartilage has also been observed in rodent sutures where a cartilaginous plate underlies the lambdoid suture, possibly forming a supportive structure on which intramembranous ossification occurs [24]. Recently, cartilage and chondrocytic markers have also been identified in sagittal sutures of transgenic mice generated with the Apert syndrome FGFR2 S252W mutation [68]. The cartilage was located at the junction of the parietal and interparietal bones which corresponds to the region from which the sutures we identified as expressing cartilage-specific genes were isolated. Importantly, we demonstrated that cartilage is present

within the osteogenic fronts, rather than underlying the sutures suggesting a functional rather than supportive role. Recently, *Sox9*, a regulator of chondrogenesis, has been shown to be upregulated during the initiation of posterior frontal suture closure in mice, along with the expression of *collagen types II and X*, followed by *collagen type I* and *osteocalcin* expression [69]. A role for endochondral ossification was therefore proposed to control fusion of this suture. Additionally, collagen types II and aggrecan have been detected in preosteogenic-condensing mesenchyme and the osteogenic fronts of developing embryonic chick heads [70]. It was proposed that, in chickens, normal intramembranous ossification includes a transient chondrogenic phase. The identification, in our study, of cartilage in lambdoid suture mesenchyme also suggests that chondrogenesis plays a role in human suture morphogenesis, particularly in the posterior skull.

Distinctive tissue-type specific gene expression differences

The analysis of gene expression in the different suture-types indicated that gene expression profiles of unfused metopic sutures were more highly correlated with the expression exhibited by fused sutures from other suture sites; specifically they showed a significantly lower expression of those genes increased in other unfused sutures (Fig. 6A, 7). This unique expression profile may help explain the earlier occurrence of metopic suture fusion during development. Unfused coronal and unfused lambdoid sutures also showed very similar expression profiles. Both these sutures are generally formed by overlapping calvarial bone fronts, in comparison to the blunt-end sutures which form the sagittal and metopic sutures [5]. These sutures are also similar in that they are a meeting of bones of two different developmental origins (mesoderm and neural crest cells) while the sagittal and metopic sutures are the meeting point of bones of one origin, either mesoderm or neural crest, respectively.

Transcription factors are key controllers of the signalling cascades activated during development. Of those transcription factors differentially expressed between fused and unfused suture tissue a majority also showed significant expression differences between suture types. Unfused sagittal sutures had a higher expression of homeobox genes *SHOX2* and *PAX5* which potentially drive osteoblast differentiation and generally had increased expression in fused sutures. The unfused sagittal sutures also showed a lower expression of *MSX2*, *SIX2*, *PITX2*, *BHLH3*, and *HLF* that were generally increased in other unfused sutures compared to fused sutures. Given the higher frequency of non-syndromic craniosynostosis in the sagittal suture we speculate that a lower expression of transcription factors associated with unfused sutures, and a higher expression of those associated with fused sutures, might leave this suture more vulnerable to premature closure.

Both unfused sagittal and unfused coronal sutures had significantly higher expression of members of the FOS (*FOS* and *FOSB*) and JUN (*JUN* and *JUNB*) oncogene families compared to unfused lambdoid sutures, particularly for coronal sutures (eg. *FOSB* was 54.4-fold increased in the coronal sutures, compared to 20.2-fold in the sagittal sutures). Through homo- and hetero-dimerisation, these proteins form the AP-1 transcription complex. Increased *Jun* and *Fos* expression occurs in prematurely fused mouse sutures induced by the application of FGF2-soaked beads [71]. It was suggested that FGF signalling increases expression of the AP-1 complex which then induces expression of osteopontin and osteoblast differentiation, resulting in premature suture closure. It is also known that the coronal and sagittal sutures are those most frequently affected in FGFR syndromic craniosynostoses. We therefore suggest that the higher expression of AP-1 transcription factor components in coronal and sagittal sutures makes them more responsive to the increased or inappropriate FGF signalling caused by gain-of-function *FGFR* mutations. However, it was also observed that unfused sagittal sutures had decreased expression of *FGFR2* compared to the coronal suture. This may explain why mutations in these genes most commonly affect the coronal suture rather than the sagittal suture.

Another important finding from our microarray analysis was that there was limited difference in whole genome expression between non-syndromic and syndromic patient samples. This was verified by qRT-PCR using 35 samples from 7 syndromic and 11 non-syndromic patients, analysing 11 genes differentially expressed between unfused and fused sutures. We note, however, that these genes were identified as being significantly differentially expressed during premature fusion by using tissue groups which contained samples from patients with different aetiologies. Thus, we specifically identified genes which were not specific to one aetiology. If these two groups of samples were analysed independently, it is likely that there may be a small proportion of genes which are differentially expressed between samples from patients with different aetiologies and these genes will be directly related to the mutation of initiation. However, in this study we were not so interested in these aetiology specific indicators, but rather the general mechanisms underlying craniosynostosis. Significantly, the results from our microarray hierarchical analysis study indicate that the genes involved in the pathogenesis of different types of craniosynostosis are more similar than may previously have been thought.

Conclusion

Through the analysis of human suture material we have identified a large number of novel differentially expressed genes, three of which, *RBP4*, *GPC3* and *C1QTNF3*, we

believe may have significant regulatory roles in the control of both suture patency and growth. Furthermore, we have identified significant gene expression differences between human sutures from different cranial sites and identified the involvement of cartilage in posterior calvarial sutures, particularly the lambdoid suture. These data open up new avenues of investigation in respect to the molecular mechanisms underlying the different responses of calvarial sutures to mutations causing craniosynostosis. This information is vital for the development of therapeutic agents to control skull growth in children with sutural defects, as well as providing clinicians with a better understanding of the developmental mechanisms operating in different sutures.

Methods

Tissue Samples

Calvarial suture samples were obtained from patients undergoing transcranial surgery for syndromic or non-syndromic craniosynostosis. Patients were genotyped for all known *FGFR1-3* and *TWIST1* mutations (Table 1) [72]. Samples used for microarray analysis (n = 16) were taken from males (n = 5) aged 3-7 months. Additional samples used for validation experiments (n = 25) and histology (n = 9) were taken from female (n = 8) and male (n = 10) patients aged 3-40 months. Additionally, six unfused suture samples were obtained from patients aged 1 day to 91 months undergoing transcranial surgery for reasons other than craniosynostosis (Table 1). Consent was provided by all guardians in line with the guidelines of the Research Ethics Committee of the Children, Youth and Women's Health Service, Adelaide, South Australia. Suture tissue was taken from prematurely fused/fusing and/or patent sutures from one or more of the sagittal, coronal, lambdoid, and metopic sutures. The site and fusion state of samples used for each analysis type are indicated in Table 1. Specimens used for microarray analysis and validation experiments were stored in RNeasy (Ambion, Austin, TX, USA) at -20°C. Specimens used for histology and immunofluorescence were fixed in formalin, decalcified with 10% EDTA, pH 7.4, by standard procedures and stored in 100% ethanol.

3DCT and MicroCT scans

Stage of fusion was confirmed by assessing 3D computer tomography (CT) images taken prior to surgery. Selected suture samples underwent MicroCT analysis to determine the degree of fusion, as previously described [73]. Briefly, tissues samples were placed in RNeasy, enclosed tightly in an acrylic tube, and analysed with a SkyScan 1072 MicroCT scanner (SkyScan, Antwerp, Belgium). 2D images were used to generate 3D reconstructions using 3D creator software (SkyScan).

Total RNA isolation

Tissues used for microarray analysis and validation experiments had the suture proper (suture mesenchyme + 3 mm bone on either side for unfused sutures, or fused bony ridge + 3 mm bone on either side for fused sutures) dissected from all specimens and the overlying pericranium was removed. Tissue samples were cut into 30-40 µg pieces for RNA extraction, snap frozen, crushed between cryogenically cooled steel blocks, and homogenised in 2 ml TRIreagent (Molecular Research Center, Cincinnati, OH, USA) using a Mini-Bead-Beater-8 (BioSpec Products, Bartlesville, OK, USA). RNA was isolated from supernatant following recommendations by Naderi *et al.* [74]. Briefly, separated aqueous phase was twice extracted with chloroform and precipitated with 1 volume isopropanol, 0.1 volume 7.5 M ammonium acetate, and 5 µg/ml linear polyacrylamide (Ambion) at -20°C overnight. Pelleted RNA was washed twice with 70% ethanol and resuspended in RNA Storage Solution (Ambion). RNA extracts from the same sample were combined. 10 µg of each combined RNA sample was purified and concentrated to greater than 300 ng/µl with phenol:chloroform:isoamyl alcohol (25:24:1) extraction. Total RNA quality was determined by analysing the integrity of the 28S and 18S ribosomal bands on a non-denaturing 1.5% agarose Tris-borate buffered gel and determining RNA purity by A260:280 ratios using UV spectroscopy.

Microarray cDNA synthesis, hybridisation, and scanning

RNA from 16 tissue samples (Table 1) was analysed using the Affymetrix expression microarray Human U133A 2.0 GeneChip platform. Concentrated total RNA was prepared for hybridisation to the GeneChips following a one-cycle target labelling protocol (Affymetrix GeneChip Expression Analysis Technical Manual). RNA was reverse transcribed into double stranded cDNA using SuperScript II (Invitrogen, Gaithersburg, MD, USA) with T7-oligonucleotides. Poly-A RNA spike-in controls were added along with 2 µg of total RNA to all cDNA reactions. Biotin labelled cRNA was prepared from the cDNA using the GeneChip IVT labelling system (Affymetrix, Santa Clara, CA, USA), with incubation at 37°C for 16 hours. 10 µg of fragmented cRNA was hybridised to each Affymetrix U133A 2.0 GeneChip. Array hybridisation, staining, and washing was carried out following manufacture's protocols using the Fluidics Station 400 (Affymetrix). Stained arrays were scanned on a GeneChip Scanner 3000 (Affymetrix) controlled by GCOS software (Affymetrix).

Microarray data analysis

CEL files containing probe intensity data were analysed in R using Bioconductor packages [75,76]. Quality control analyses were carried out on probe level model (PLM) normalised samples. Normalised un-scaled standard error (NUSE) box plots, Mbox plots, and RNA degradation

plots were analysed [77]. For statistical analyses probe intensity data were normalised using the GeneChip Robust Multichip Average (GCRMA) algorithm, which has been shown to provide a good balance between accuracy and precision [78]. Diana-divisive hierarchical clustering was used to cluster the microarray samples based on whole genome expression values. To identify differential gene expression the Limma package was used to fit linear models to the data, incorporating an empirical Bayes modification of the standard errors [79]. False discovery rate adjustment of P-values was performed to account for multiple testing [80]. Correlation plots and hierarchical trees were generated using cluster packages available in R. Gene ontology over-representation was analysed using GOTree Machine, normalising to the U133A 2.0 gene set, with significance set at $P < 0.01$ [81,82]. Gene set enrichment analysis was carried out using GSEA v 2.01, comparing the ranked list of 2-fold differentially expressed probe sets to gene sets c1-c4 (v2.symbols.gmt) [83,84]. Gene set exclusion was set at min = 4, max = 500, with 1000 weighted permutations executed. A 3-way contrast matrix was created for the unfused suture comparisons: coronal-sagittal, lambdoid-sagittal, and lambdoid-coronal. Using the Limma package a Venn diagram was produced for those genes identified to be differentially expressed using a nested F-test approach which gives particular attention to genes which are differentially expressed ($P < 0.01$) under 2 or more conditions. To control for patient-specific effects due to the small sample size of this comparison, we performed a set of separate pair wise comparisons using a matched pairs design. In this case we first restricted analysis to patients with a sample from each of the sutures of interest, and then performed analysis on the within patient differences. A matched-pairs matrix was constructed for an unfused coronal-lambdoid comparison using samples from patients #36 and #46 and a coronal and sagittal comparison using samples from patients #36 and #58. A t-test was performed between unfused lambdoid and sagittal samples from patients #36, #46 and #58. A linear model incorporating an empirical Bayes modification was applied to each matched pairs comparison. To identify genes differentially expressed in unfused sagittal sutures, the intersection of the 3-way and matched pairs coronal-sagittal comparison and lambdoid-sagittal comparisons was found.

Realtime quantitative RT-PCR (qRT-PCR)

Total RNA was reverse transcribed into cDNA using SuperScript III (Invitrogen). Two micrograms of RNA was added to 40 µl total volume reactions which were carried out following the manufacturer's protocol. In addition to the patient RNA samples, a calibrator RNA sample which was used to standardise absolute qRT-PCR results, was transcribed into cDNA, column purified (QIAquick PCR purification kit, Qiagen, Clifton Hill, VIC, Australia) and

quantified by UV spectroscopy. cDNA from all samples was diluted 1/3 and 1/120 with TE (pH 8) and supplemented with herring sperm DNA to 1 ng/ μ l. The 1/120 dilutions were used for the amplification of the 18S rRNA gene and the 1/3 dilutions were used for the analysis of all other genes. Absolute quantification was carried out using standard curves generated by serial dilution of target amplicon-containing plasmids (pGEM-T easy, Promega, Annandale, NSW, Australia), to cover up to 5 logs of amplicon copy number per microlitre. All primers were designed to target the same sequence as the microarray probes, to overlap exon-exon junctions, and to have a melting temperature of 60°C (Additional file 13). Real-time reactions were carried out using SYBR green (Applied Biosystems, Foster City, CA, USA) on a ABI Prism 7000 Sequence Detection System (Applied Biosystems). Twenty microlitre reactions contained 2 μ l of cDNA and 0.4 μ M each primer. PCR amplification followed a two step cycling protocol; 10 min denaturation at 95°C followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Melting curve analysis was conducted to confirm specific amplicon amplification. Patient reactions were performed in triplicate and standard curve points in duplicate. ABI Sequence Detection Software version 1.2 was used to determine sample Ct values, with the same threshold set for all reactions. Absolute copy number values calculated from standard curves were normalised to a calibrator cDNA sample (1 ng was used for RT-PCR) by calculating a ratio of the patient 18S Ct to that of the calibrator sample 18S Ct. Differences between samples were analysed for log₁₀ transformed data by Student's t-test, with significance set at $P < 0.05$.

Western blot analysis

Whole tissue protein was isolated from the TRIreagent organic phase separated during RNA extraction following the manufacturer's instructions and reconstituting in 10 M urea. Protein was quantified using the Bio-Rad Protein Assay (Bio-Rad, Regent Park, NSW, Australia) and 25 ng of total protein was resolved by 10% SDS-PAGE and transferred to a Hybond-C nitrocellulose membrane (Amersham, North Ryde, NSW, Australia). Membranes were blocked with Odyssey Blocking Buffer (LI-COR Biosciences, Lincoln, NE, USA) diluted 1:1 in TBS (50 mM TRIS pH 7.5, 150 mM NaCl) for 45 min. Antibodies were diluted 1:1 in blocking buffer with 0.1% Tween-20. Blocked membranes were probed with either mouse monoclonal anti-collagen type I (COL1, 1:100, Calbiochem, Alexandria, NSW, Australia) or goat anti-mouse CORS26/C1qTNF3 (1:50, R&D Systems, Minneapolis, MN, USA) and incubated overnight at 4°C. COL1-probed membranes were double probed with rabbit polyclonal anti-human retinol-binding protein (RBP4, 1:20000, DAKO, Botany, NSW, Australia) for 1 h at room temperature (RT). Membranes were washed three times in TBST (50

mM TRIS pH 7.5, 150 mM NaCl, 0.1% Tween-20) following primary antibody incubation. Antibody binding to double probed membranes was detected by infrared emission using goat anti-rabbit Alexa Fluor 680 (1/20000, Molecular probes, Eugene, OR, USA) and goat anti-mouse IRDye 800 (1:15000, Rockland, Gilbertsville, PA, USA). Antibody binding to single probed membranes was detected with donkey anti-goat Alexa Fluor 680 (1:10000, Molecular Probes). Protein bands were detected and quantified using the Odyssey infrared imaging system (LI-COR Biosciences). Double probed membranes were stripped with low pH stripping buffer (25 mM glycine-HCL pH 2, 1% (w/v) SDS) for 30 min at RT, followed by washing in TBST. Stripped membranes were blocked in 5% skimmed milk in TBST for 30 min. Membranes were probed with primary sheep anti-human glypican 3 (GPC3, 1:2000, R&D Systems), followed by rabbit anti-sheep horseradish peroxidase-conjugated antibody (1:2000, Chemicon) and Immobilon Western Substrate (Millipore, North Ryde NSW, Australia). Antibodies for GPC3 detection were diluted in 5% skimmed milk in TBST and incubated for 1 h at RT. For densitometry, GPC3 blots were scanned using the Odyssey imaging system (LI-COR Biosciences).

Immunofluorescence confocal microscopy

Fixed and decalcified specimens were dehydrated through a graded ethanol series and embedded in paraffin. Sections were cut to 3 μ m thickness, mounted on 3-aminotriethoxysilane (APES)-coated slides and incubated for 16 h at 60°C, followed by 7 h at 37°C. Sections were deparaffinised and rehydrated in distilled H₂O for 5 min. Antigen retrieval was carried out using TEG buffer (TRIS-EGTA, pH 9.0) for RBP4 and COLX and TRIS-HCL buffer (pH 1.0) for GPC3. All sections were incubated at 60-70°C overnight with constant stirring. Slides were cooled and washed in 1 \times PBS (pH 7.4) before incubating in blocking buffer (0.3% casein, 0.1% Tween-20, in 1 \times PBS pH 7.4) for 15 min. Rabbit polyclonal anti-human RBP4 (1:2000, DAKO), sheep anti-human GPC3 (1:50, R&D Systems), and mouse monoclonal anti-collagen type X (COLX, 1:500, Sigma-Aldrich, Castle Hill, NSW, Australia) primary antibodies were incubated for 1 h, followed by washing in 1 \times PBS and Tween 20 (0.1%). Sections were incubated with corresponding secondary antibodies (goat anti-rabbit Alexa Fluor 488, donkey anti-sheep Alexa Fluor 488, goat anti-mouse Alexa Fluor 488) for 1 h at RT followed by washing. All antibodies were diluted in 1 \times PBS. Sections were coverslipped using Pro-Long Gold antifade (Invitrogen) and viewed with a Leica TCS 4D confocal laser scanning microscope (Leica Laser Technology, Heidelberg, Germany). Secondary antibodies were excited with a 488-nm laser and fluorescent light detected using a FITC band pass 520-560 nm barrier filter. The controls were prepared in the absence of the primary

and the secondary antibodies, and with both antibodies but without antigen retrieval, and were negative in all cases.

Histology and cartilage detection

Decalcified formalin fixed specimens were sectioned (3 μ m), mounted onto APES-coated slides, and incubated at 60°C for 16 h. Deparaffinised and rehydrated sections were stained with 1% Alcian blue in 3% aqueous acetic acid (pH 2.5) to detect the presence of cartilage, or hematoxylin and eosin for tissue structure, and mounted in Depex (Sigma-Aldrich). Sections were imaged using a brightfield microscope (Carl Zeiss Jena, Jena, Germany) equipped with a DFC480 digital camera (Leica Microsystems).

Authors' contributions

AKC performed RNA and protein isolation and quantification, RNA processing and microarray scanning and data mining, realtime quantitative RT-PCR and Western blot validation experiments, confocal immunofluorescence and histological analysis of suture tissues, and drafted the paper. Immunofluorescence and histological stainings were reviewed and discussed by AKC, IH, PA, and BP. PJA collected the suture samples and performed decalcification and fixation of specimens. CRW coordinated microarray quality control, data mining, and statistical analysis of quantitative RT-PCR validation experiments. BP assisted with drafting of the paper. AKC, AVD, BP, PA, PM, and IH participated in study's design and coordination. All authors have reviewed the manuscript. BP, IH, and AKC were the final editors of the manuscript.

Additional material

Additional file 1

Microarray quality control RNA digestion plot. RNA digestion plot, for the 16 RNA samples hybridised to Affymetrix Human expression U133A 2.0 GeneChip microarrays. RNA digestion plot compares expression intensity for all probes which bind sequences in order of 5' to 3' along a transcript, for all probe sets on the GeneChip.

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Additional file 2

Microarray quality control NUSE box plots. Normalised unscaled standard error (NUSE) box plots for the 16 RNA samples hybridised to Affymetrix Human expression U133A 2.0 GeneChip microarrays. NUSE box plot shows a ratio of the NUSE for each probe set compared to a median value of the NUSEs across all arrays.

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Additional file 3

Microarray quality control Mbox plots. Mbox plots for the 16 RNA samples hybridised to Affymetrix Human expression U133A 2.0 GeneChip microarrays. M box plot shows the range of fold change (M) for each probe set expressed by one sample compared to the mean expression of that probe set across all samples analysed.

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Additional file 4

Hierarchical cluster based on whole genome expression. Diana divisive hierarchical cluster of the 16 tissue samples analysed based on the expression intensity of all probe sets on the microarray.

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Additional file 5

Genes with > 2-fold differential expression between sutures. List of the top 829 differentially expressed genes with > 2-fold differential expression between fusing/fused and unfused suture tissue. A positive value indicates higher levels in fused sutures and a negative value indicates higher expression in unfused sutures.

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Additional file 6

Enrichments plots generated by GSEA analysis for selected significantly correlated datasets. The colour bar depicts phenotype correlation based on ranking metric scores. Red indicates those genes with increased expression in fused sutures and blue indicates those genes with increased expression in unfused sutures. Black bars represent genes ordered by their ranking within the 2-fold differentially expressed gene list between fused/fusing and unfused sutures.

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Additional file 7

H&E analysis of suture tissue. A large number of white blood cells were observed in the calvarial tissue. The majority of cells appear as lymphocytes (*).

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Additional file 8

Genes identified to be differentially expressed ($P < 0.01$) by matched pairs analysis between unfused coronal and sagittal sutures. A positive value indicates higher expression levels in coronal sutures and a negative value indicates higher expression in sagittal sutures.

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Additional file 9

Genes identified to be differentially expressed ($P < 0.01$) by matched pairs analysis between unfused lambdoid and sagittal sutures. A positive value indicates higher expression levels in lambdoid sutures and a negative value indicates higher expression in sagittal sutures.

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Additional file 10

Genes identified to be differentially expressed ($P < 0.01$) by matched pairs analysis between unfused coronal and lambdoid sutures. A positive value indicates higher expression levels in coronal sutures and a negative value indicates higher expression in lambdoid sutures.

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Additional file 11

Syndromic and non-syndromic sutures are not significantly different. P -values from Student's t -test comparing gene expression between syndromic and non-syndromic samples from for each stage of development. Samples from different suture sites were combined for analysis.

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Additional file 12

qRT-PCR results for the additional genes not shown in Figure 6A. The long isoform of C1QTNF3 had limited to no differential expression between unfused, fusing, and fused suture from the different sites and was therefore not the highly significantly differentially expressed isoform. COL8A2 and COL3A1 had increased expression in unfused compared to fused sutures, except in the sagittal suture.

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Additional file 13

Primers used for realtime qRT-PCR.

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In Vitro Differentiation of Human Calvarial Suture Derived Cells With and Without Dexamethasone Does Not Induce in Vivo-Like Expression

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Osteogenic supplements are a requirement for osteoblastic cell differentiation during in vitro culture of human calvarial suture-derived cell populations. We investigated the ability of ascorbic acid and β -glycerophosphate with and without the addition of dexamethasone to stimulate in vivo-like osteoblastic differentiation. Cells were isolated from unfused and prematurely fused suture tissue from patients with syndromic and non-syndromic craniosynostosis and cultured in each osteogenic medium for varying lengths of time. The effect of media supplementation was investigated with respect to the ability of cells to form mineralised bone nodules and the expression of five osteodifferentiation marker genes (*COL1A1*, *ALP*, *BSP*, *OC* and *RUNX2*), and five genes that are differentially expressed during human premature suture fusion (*GPC3*, *RBP4*, *CIQTNF3*, *WIFI* and *FGF2*). Cells from unfused sutures responded more slowly to osteogenic media but formed comparable bone nodules to fused suture-derived cells after 16 days of culture in either osteogenic media. However, gene expression differed between unfused and fused suture-derived cells, as did expression in each osteogenic medium. When compared to expression in the explant tissue of origin, neither medium induced a level or profile of gene expression similar to that seen in vivo. Overall, our results demonstrate that cells from the same suture that are isolated during different stages of morphogenesis in vivo, despite being de-differentiated to a similar level in vitro, respond uniquely and differently to each osteogenic medium. Further, we suggest that neither cell culture medium recapitulates differentiation via activation of the same genetic cascades as occurs in vivo.

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The calvarial suture complex is a heterogeneous tissue comprised of osteoblastic mesenchyme, osteoprogenitor cells, preosteoblasts, osteoblasts and osteocytes, as well as cells from other developmental lineages. Explant culture of osteoblastic cells derived from human calvarial suture tissue provides an in vitro model to study the processes of bone formation which includes progenitor cell proliferation and osteoblast differentiation coupled via an inverse relationship (Owen et al., 1990; Malaval et al., 1999). Furthermore, the culturing of cells derived from unfused and prematurely fused sutures from patients with craniosynostosis facilitates phenotypic and genetic investigations of pathologic processes such as deregulation of proliferation, differentiation, or other processes, such as apoptosis, which may result in this craniofacial developmental abnormality (for review, see Opperman, 2000; Morriss-Kay and Wilkie, 2005). For the results from such experiments to be appropriately translated to the clinical situation, the in vitro system used to investigate these patient-derived cells must therefore relate as close as possible to the in vivo environment from which they were obtained.

Osteoblast development is generally subdivided into three stages: (1) proliferation, (2) extracellular matrix maturation, and (3) mineralisation (Owen et al., 1990). In vitro analysis of the different developmental stages is conducted using different culture conditions: proliferation is analysed under minimal medium, while differentiation and matrix mineralisation are

analysed using media supplemented with various osteogenic factors. The capacity of cells to differentiate is evaluated by their ability to deposit a collagenous extracellular matrix (ECM) and to form bone nodules consisting of multiple layers of cells in a mineralised extracellular matrix. The stage of differentiation of these cells is estimated through an analysis of temporally expressed differentiation-associated genes such as *runx-related transcription factor 2* (*RUNX2*), *collagen type I* (*COL1A1*), *alkaline*

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phosphatase (ALP), bone sialoprotein (BSP), and osteocalcin (OC) (for review, see Aubin and Triffitt, 2002). *RUNX2* and *COL1A1* are the earliest markers of immature osteoprogenitor cells, ALP is expressed in mature osteoprogenitors/preosteoblasts and mature osteoblasts, and is down-regulated when mineralisation is well progressed. BSP is expressed transiently twice, initially in primitive osteoprogenitor cells, and then during osteoprogenitor differentiation after ALP up-regulation. Finally, OC is expressed in post-proliferative osteoblasts concomitantly with mineralisation (Candelieri et al., 1999; Liu et al., 2003).

As differentiation of osteoprogenitor cells is dependent upon the formation of an extracellular matrix (Franceschi and Iyer, 1992), the standard differentiation medium incorporates ascorbic acid, which is a cofactor in collagen matrix synthesis, and organic phosphate (e.g. β -glycerophosphate), to promote mineralisation (Nefussi et al., 1985; Franceschi, 1992). The selection of an appropriate minimal media is also important due to their basal concentrations of β -glycerophosphate (Gerber and Gwynn, 2001). A primitive calvarial-derived osteoprogenitor cell population also exists in culture which only differentiates through the addition of specific inductive stimuli such as glucocorticoids (e.g. dexamethasone), other steroids (e.g. progesterone), and other factors such as bone morphogenetic proteins (Turksen and Aubin, 1991; Hughes et al., 1995; Ishida and Heersche, 1997).

The use of these supplements creates an artificial environment which may induce osteoblastic differentiation of particular cell populations, or matrix mineralisation that mimics in vivo bone formation. However, it is unlikely that these processes will occur through the activation of the complex network of pathways that interact in vivo during calvarial osteoblast differentiation (for review, see Rice et al., 2003; Morriss-Kay and Wilkie, 2005). Rather, the phenotype is more likely to be derived through specific activation of a restricted number of pathways not necessarily representative of the in vivo situation. Indeed, it is known that dexamethasone affects *FGFR1* gene expression differently in vitro and in vivo (Meisinger et al., 1996).

We have previously shown that after explant culture, irrespective of the origin of calvarial explant tissue, whether it be unfused human sutures which are predominantly comprised of mesenchyme and osteoprogenitor cells, or prematurely fused human calvarial sutures which are predominantly comprised of differentiated osteoblasts/osteocytes, these cells immediately lose their in vivo specific expression profile and de-differentiate to a similar level (Coussens et al., 2008). Under minimal medium conditions, the gene expression of these different cell populations essentially stabilises after the first passage and an in vitro specific gene expression profile similar to that of undifferentiated osteoblastic cells is adopted. Thus, the degree to which the commonly used media supplements restore the expression profile of these de-differentiated cells to that seen in vivo is an important question for osteogenic-cell biologists to consider.

Here, we have analysed the effect of two osteogenic media (standard differentiation medium (DMEM), which has relatively low β -glycerophosphate levels (Gerber and Gwynn, 2001) containing ascorbic acid and β -glycerophosphate with, and without, dexamethasone) on the phenotype and gene expression profiles of human calvarial bone explant cells isolated from both unfused and prematurely fused calvarial sutures from patients with syndromic and non-syndromic craniosynostosis. We compared the induced gene expression profiles between both cell types and also determined if differential expression profiles which are observed between unfused and fused suture tissues in vivo are maintained in vitro. Finally, as fused sutures represent a fully differentiated tissue type, we also compared the levels of gene expression between explant tissues and corresponding explant cells grown in

osteogenic supplements to determine the ability of osteogenic supplements to induce in vivo-like differentiation. Gene expression was analysed for five common differentiation marker genes. In addition, five genes were analysed that we had previously identified to exhibit high levels of differential expression between unfused and prematurely fused human calvarial tissues, namely *glypican 3* (*GPC3*), *retinol binding protein 4* (*RBP4*), *Clq and tumor necrosis factor related protein 3* (*CIQTNF3*), *WNT inhibitory factor 1* (*WIF1*), and *fibroblast growth factor 2* (*FGF2*) (Coussens et al., 2007). We show that despite stimulating the formation of a mineralised ECM, osteogenic supplements added to minimal medium generally produce a limited modulation of gene expression. Furthermore, the induced expression profiles for each medium were unique and also differed depending on the nature of the tissue from which the de-differentiated cells were isolated (e.g. fused or unfused sutures). Finally, none of these induced profiles were equivalent to those observed in the tissues of origin.

Materials and Methods

Suture samples

Calvarial suture samples were obtained from three patients undergoing transcranial surgery for craniosynostosis. Consent was provided by all guardians in line with the guidelines received from the Research Ethics Committee of the Children, Youth and Women's Health Service, Adelaide, South Australia. Patients were previously genotyped for all known mutations in craniosynostosis-causing genes *FGFR1-3* and *TWIST1* (Anderson et al., 2007). Suture tissue was taken from prematurely fused coronal and lambdoid sutures from a patient (male, 7 months) diagnosed with Apert syndrome (AP) and carrying an *FGFR2* Ser252Trp mutation. Samples of unfused coronal and lambdoid sutures were taken from a patient (male, 7 months) with non-syndromic sagittal synostosis (NS) who was negative for *FGFR* and *TWIST1* mutations. Samples were sectioned into two parts on removal. Specimens used for cell culture were placed in Ringers solution for up to 4 h until processed. Specimens used for tissue RNA extraction were stored in RNAlater (Ambion, Austin, TX). To determine if the phenotypic differences observed between the cells derived from non-syndromic and syndromic samples were due to the different aetiologies of craniosynostosis, cells were cultured from unfused and prematurely fused sections of the same lambdoid suture from an additional patient (male, 4 months) with Pfeiffer syndrome (PF) harbouring an *FGFR2* splice mutation. Cellular mineralisation was similarly assessed for cells from this patient, however no tissue RNA was obtained to facilitate gene expression comparisons. The suture complex (suture mesenchyme plus 3 mm of bone on either side for unfused sutures, or fused bony ridge plus 3 mm of bone on either side for fused sutures) was dissected from all specimens and the overlying pericranium removed.

Suture cell culture

Human calvarial suture cells were obtained by collagenase digestion and explant culture following the method described by de Pollack et al. (1996). Briefly, dissected suture samples were minced into 1 mm fragments and incubated in 0.25% collagenase for 2 h at 37°C. Samples were centrifuged and supernatant removed. Following three washes in PBS, samples were plated at 5 bone fragments per well, in 12-well plates, and cultured in minimal medium in a humidified atmosphere of 5% CO₂ kept at 37°C. Minimal medium consisted of high glucose Dulbecco's modified essential medium (DMEM, Invitrogen Life Technologies, Gaithersburg, MD) supplemented with L-glutamine (584 mg/L), 10% foetal calf serum, and 1% antibiotics (penicillin 100 IU/ml, streptomycin 100 μ g/ml). Upon confluence, cells were plated in T25 flasks and labelled P1. Medium was changed every 2 days. Cells

were passed to P3 to obtain sufficient cells before being frozen down and stored in liquid nitrogen. Frozen cells were brought up at P4, plated under minimal medium into 24-well plates at a density of 2×10^4 cells/well. Samples were plated in triplicate, for each of the three media, for RNA extraction (9 wells per sample per time point) and in duplicate, for each of the three media, for mineralisation assays (6 wells per sample per time point). Passage-four cells were used for all experiments to ensure that any tissue-specific expression was lost, thus enabling the precise delineation of the effect of each supplement on gene expression. No difference in mineralisation potential or gene expression between P1 and P4 cells has been observed previously (Owen et al., 1990; Gerber and Gwynn, 2001; Coussens et al., 2008). At confluence (day 6), medium was replaced with either minimal medium (Min), standard osteogenic medium (OM; minimal medium supplemented with 0.05 mM ascorbic acid and 10 mM β -glycerolphosphate), or osteogenic medium supplemented with 100 nM dexamethasone (OM + D). Media were changed every 3 days. RNA was extracted at day 16 (i.e. 10 days post-supplementation) for all samples and at day 22 (i.e. 16 days post-supplementation) for coronal suture-derived cells.

Extracellular matrix and bone nodule assay

Matrix formation and mineralisation was measured at days 7, 16 and 22. Cells were washed in distilled H_2O followed by staining for mineralisation using the von Kossa method and then for collagenous extracellular matrix formation using the van Gieson method, followed by fixation in 70% ethanol.

Total RNA isolation and cDNA synthesis

Total RNA was isolated from P4 cells, after washing with PBS and homogenisation in 500 μ l per well of TRI Reagent (Molecular Research Centre, Inc., Cincinnati, OH), following the manufacturer's protocol. Total RNA was extracted from tissue samples as previously described (Coussens et al., 2007). Briefly, tissue samples stored in RNAlater were thawed on ice and cut into 30–40 mg pieces. Individual pieces were snap frozen, crushed, and homogenised in 2 ml TRI Reagent using a Mini-Bead-Beater-8 (BioSpec Products, Bartlesville, OK). RNA was isolated from supernatant according to Naderi et al. (2004) using 5 μ g/ml linear polyacrylamide (Ambion) and isopropanol precipitation. RNA extracts from the same tissues were combined and 10 μ g of each combined RNA was purified and concentrated with a phenol/chloroform/isoamyl alcohol (25:24:1) extraction. RNA was reverse transcribed into cDNA using SuperScript III (Invitrogen Life Technologies) following the manufacturer's protocol. In addition to the experimental RNA samples, a calibrator RNA sample which was used to standardise absolute quantitative RT-PCR (qRT-PCR) results, was transcribed into cDNA, column purified (QIAquick PCR purification kit, Qiagen, Clifton Hill, VIC) and quantified by UV spectroscopy. cDNA from all samples was diluted 1/3 and 1/120 with TE (pH 8.0) supplemented with herring sperm DNA to 1 ng/ μ l (Promega, Annandale, NSW, Australia). The 1/120 dilutions were used for the amplification of the *18S* rRNA gene and the 1/3 dilutions were used for the analysis of all other genes.

Real-time absolute qRT-PCR

Absolute quantification was carried out using standard curves generated by 10-fold serial dilution of target amplicon-containing plasmids (pGEM-T Easy Vector, Promega) which covered at least 5 logs of amplicon copy number. Primers were designed over exon boundaries using Primer Express software (Applied Biosystems, Foster City, CA) for *COL1A1*, *ALP*, *BSP*, *OC*, *RUNX2*, *GPC3*, *RBP4*, *CIQTNF3*, *WIF1*, *COL3A1*, *FGF2* and the reference gene *18S* rRNA (Table 1). All primers were designed over exon boundaries and amplicons were designed with a melting temperature of 60°C. Reactions were carried out using SYBR green (Applied Biosystems) on a Prism 7300 Sequence Detection System (Applied Biosystems). Each tissue-derived cDNA was analysed in triplicate reactions, cDNAs generated from three independent experiments for each cell culture treatment were analysed using one reaction each, and standard curve points were analysed in duplicate reactions. Melting curve analysis was conducted to confirm specific amplicon amplification without genomic DNA contamination. Absolute copy number values calculated from standard curves were normalised to a calibrator cDNA sample (1 ng was used for qRT-PCR) by calculating a ratio of the cycle threshold (Ct) for each experimental-sample *18S* to that of the calibrator-sample *18S* Ct.

qRT-PCR statistical analysis

Triplicate expression results from each cell culture treatment or tissue were combined to give a mean absolute expression \pm SEM. Student's *t*-test was used to test for significance between \log_{10} transformed expression levels in tissue or cells grown in osteogenic media and cells grown under minimal media. Significance was taken as $P < 0.05$ and a Bonferroni multiple comparison correction was applied to *P*-values. Coronal day 16 minimal medium comparisons were corrected for 4 comparisons (tissue, OM, OM + D, minimal day 22; $P < 0.0127$) and coronal day 22 and lambdoid day 16 comparisons were corrected for 3 comparisons (tissue, OM, OM + D; $P < 0.0170$).

Results

Different osteogenic media stimulate unique mineralisation phenotypes

Collagenous ECM and bone nodule formation was assessed in primary cells derived from unfused and fused sutures from three patients: one with non-syndromic (NS) and two with syndromic (AP and PF) craniosynostosis and cultured in three different media. At day 7 no difference between media was observed for all cell populations (data not shown). At day 16, mineralised ECM formation was observed for cells grown under both osteogenic media (OM and OM + D), but not those under minimal medium (Fig. 1). The only exception was fused coronal cells from patient AP which under OM media produced a collagenous ECM but failed to mineralise. Both unfused and fused suture-derived cell populations displayed greater matrix mineralisation in OM + D compared to OM. However, explant cells from fused sutures had greater ECM and bone nodule formation compared to cells from unfused sutures.

TABLE 1. Primers used for real-time quantitative RT-PCR

Primer set	Forward (5'–3')	Reverse (5'–3')	Amplicon (bp)
ALP	CGTGGCTAAGATGTCATCATGTT	TGGTGAGCTGACCCTTGA	90
BSP	TTTCTGCTACAACACTGGGCTATG	TTGAGAAAGCACAGGCCATTC	102
CIQTNF3	CTTTGGAGGCGAGCTCATCTATTG	CCGGTTTGTGGAGACTCCATG	95
COL1A1	CGAAGACATCCCACCAATCAC	TTGTCCGAGACGCAGATCC	99
COL3A1	TCAACACTCTTTATGATAACAACACTGTG	TCGATTTTGACTATTTCAGAAAGAAAGG	119
FGF2	AGAAGAGCGACCCTCACATCA	GCCAGGGTAACGGTTAGCACAC	91
GPC3	CTTCTTTGCGAGAATGGCCTATG	GGTTGTGAAAGGTGCTTATCTCG	101
OC	CCACCGAGACACCATAGAGGC	CCAGCGATGCAAGTGCG	69
WIF1	CCTCTGTTCAAAGCTGTCTGC	GTGTCTTCCATGCCAACCTTC	100
18S	TATCAGATCAAAACCAAC	CGAAAGTTGATAGGGCAG	151

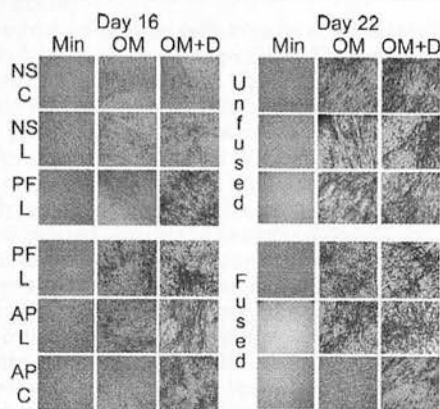


Fig. 1. Von Kossa (mineralisation, black) and van Gieson (ECM, orange) staining of cells derived from unfused and fused coronal (C) and lambdoid (L) sutures from three patients: NS, non-syndromic sagittal synostosis; PF, Pfeiffer syndrome (*FGFR2* Exon 9 splice mutation); AP, Apert syndrome (*FGFR2* S252W). Cells were grown in minimal medium (Min), standard osteogenic medium (OM; 0.05 mM ascorbic acid and 10 mM β-glycerophosphate) and standard osteogenic medium with 100 nM dexamethasone (OM + D). Unfused suture-derived cells exhibit less mineralisation than fused suture-derived cells at day 16, but show similar matrix mineralisation at day 22, irrespective of patient of origin and aetiology. Lambdoid suture-derived cells respond better to osteogenic supplements than coronal-derived cells. Fused coronal suture-derived cells only mineralised in the presence of dexamethasone, while all other cells mineralised in both osteogenic media at day 22. No mineralisation was observed under minimal medium. Images are sample representations of three individual cultures (see Supplementary Material Fig. 1).

Importantly, this difference was also seen between cells isolated from unfused and fused sections of the same sutures from a Pfeiffer syndrome patient (PF) with an *FGFR2* mutation. Therefore, it is likely that the differential response observed in Figure 1 between unfused and fused suture-derived cells are due to cellular differences related to the developmental stage of the tissue from which the cells were derived (i.e. suture

mesenchyme vs. mineralised bone) rather than to any background genetic differences. It was also noted that at day 16 cells from both unfused and fused lambdoid sutures responded better to media supplements than those from coronal sutures. This is consistent with our previous findings that suture tissues obtained from different anatomical sites in humans display different gene expression profiles (Coussens et al., 2007). Despite the formation of an ECM, unfused coronal suture cells did not form nodules in either media and fused cells only formed mineralised bone nodules in OM + D (Fig. 1). Cells were therefore cultured for a further 6 days to determine if coronal cells would mineralise in OM. After 22 days in culture (16 days of osteogenic media supplementation), unfused coronal suture cells mineralised in both OM and OM + D but not to the same extent as unfused lambdoid suture cells. Furthermore, cells from fused coronal sutures remained unmineralised under OM, despite the formation of an extensive collagenous ECM, with mineralisation again only occurring under OM + D. It would therefore seem that this population of fused suture cells represents the more immature osteoprogenitor-like cell population that requires steroid stimulation for mineralisation, as has been previously described (Turksen and Aubin, 1991).

Each osteogenic media induces unique gene expression profiles

While there was only minimal phenotypic difference between cells grown in the different osteogenic media, the gene expression induced by each media was largely different. Moreover, the induced gene expression profiles differed between cells isolated at different stages of development and from different cranial sites (Table 2). In cells derived from unfused coronal and lambdoid sutures, after 16 days of culture (10 days post-supplementation), both OM and OM + D media significantly decreased expression of *FGF2*, *OC* and *WIF1* compared to minimal medium, and there was a further decrease in *FGF2* and *OC* at day 22. There was a significant increase in expression of *GPC3* at day 16 which was further increased at day 22. Contrastingly, OM alone increased expression of *CIQTNF3* and *BSP*, while OM + D medium decreased expression of *CIQTNF3*, *RBP4*, and *COL1A1* and increased expression of *ALP*. Interestingly, neither supplement

Table 2. Effect of supplementation on *in vitro* gene expression compared to minimal medium

Medium ^a	UNFUSED				FUSED			
	OM		OM+D		OM		OM+D	
	d16	d22	d16	d22	d16	d22	d16	d22
RUNX2	=	=	=	=	C=	L↑	↑	↑
COL1	=	↑	↓	↓	=	=	↓	=
ALP	=	=	↑	↑	↓	=	↑	↑
BSP	↑	↑	=	=	↑	↑	↑	↑
OC	↓	↓	↓	↓	=	=	C↓	L↑
FGF2	↓	↓	↓	↓	↓	↓	↓	↓
CIQTNF3	↑	↑	↓	↓	↑	↑	C=	L↓
GPC3	↑	↑	↑	↑	=	↑	↑	↑
RBP4	=	↓	↓	↓	C↓	L=	=	=
WIF1	↓	=	↓	=	=	=	C↑	↑

Unfused cells are from patient NS and fused suture cells are from patient AP. Fused coronal (C) cells did not mineralise and have a different expression profile to fused lambdoid (L) cells.
^aOM, standard differentiation media; OM+D, standard differentiation media plus dexamethasone
↑, increased expression; ↓, decreased expression; =, no change in expression

affected the expression of *RUNX2* compared to that under minimal medium.

For fused suture-derived cells, in general, a greater difference in gene modulation was induced by OM + D medium compared to standard osteogenic medium (Table 2). Furthermore, fused suture derived cells from coronal and lambdoid sutures from the same patient had differing responses at day 16 to each medium for 5 genes analysed (*RUNX2*, *CIQTNF3*, *RBP4*, *WIFI*, *OC*). The only consistent patterns observed for both media for both cell types was the similar decrease in expression of *FGF2* as was seen in unfused sutures and an increase in expression of *BSP* (at both time points), *RUNX2* (day 22) and *GPC3* (day 22). This up-regulation of *RUNX2* was in contrast to the lack of response of *RUNX2* in unfused cells to both osteogenic media. The only other similarity between unfused and fused cells was the consistent difference between media seen for *CIQTNF3* expression, with increased expression induced by OM and decreased, or unchanged (fused coronal cells) expression induced by OM + D. Other differences seen between osteogenic media in fused suture cells were decreased expression of *COL1A1* and *OC* (only in coronal cells at day 16), and increased expression of *ALP*, *WIFI* (only in coronal cells), and *OC* (only in lambdoid cells at day 16) caused by OM + D, but not OM.

Neither media induced in vivo-like expression levels

We have previously shown that for short-term culture under proliferative conditions in vitro gene expression is severely modulated compared to that in vivo and that after the first passage calvarial suture cells adopt a relatively stable in vitro specific expression profile that is maintained until at least passage 4, irrespective of the fusion state of the explant tissue (Coussens et al., 2008). We therefore wanted to see if common differentiation media stimulate expression back to the levels observed in vivo after long-term culture. Only fused suture tissue-derived cells were compared to their corresponding tissues as fused sutures represent a fully differentiated cell population unlike unfused suture tissues which would have cells of varying stages of differentiation.

In this study, after long-term culture in minimal medium (16 and 22 days) we observed a similar dramatic modulation of gene expression compared to that in vivo (Fig. 2). Most genes were significantly down-regulated under minimal medium at both time points. The exceptions were *FGF2* which was significantly up-regulated at both time points and *CIQTNF3* which displayed in vivo expression levels in fused coronal cells at day 16 and was significantly up-regulated compared to in vivo levels in fused lambdoid cells at day 16 and in fused coronal cells at day 22. *GPC3* also showed expression levels similar to in vivo expression levels after 22 days in culture in minimal medium (Fig. 2).

Surprisingly after long-term culture in either osteogenic media a number of key differentiation markers were still expressed at levels markedly different from that in vivo. For example, while *BSP* and *OC* expression was induced towards that seen in vivo by both osteogenic media, expression levels still remained 100- to 1,000-fold less. *ALP* was only induced to similar in vivo levels by OM + D, while expression under OM remained up to 10-fold less than in vivo levels and at day 16 was lower than that under minimal media for both coronal and lambdoid suture-derived cells. Another striking observation was that the greater than 100-fold increase in *FGF2* observed under minimal media was only slightly reduced by both osteogenic media, remaining about 100-fold higher than in vivo levels. One of the only genes that reached in vivo levels under both media was *RUNX2*, but this was only achieved at day 22.

In vivo differential expression patterns are not maintained in vitro

Although neither osteogenic media was able to recapitulate the level of gene expression observed for fused suture tissue in vivo, we were interested in investigating whether differential expression patterns observed in vivo between unfused and fused sutures tissues can be recapitulated in vitro using either osteogenic medium. We therefore then compared expression between unfused and fused suture-derived cells grown in both OM and OM + D for 10 days (day 16), to the differential expression patterns observed for these two cell populations in vivo. day 16 was chosen as this was the time point in which the greatest phenotypic difference in mineralisation was observed between the two cell populations; little mineralisation being observed in unfused cells compared to fused sutures cells, reflecting the phenotype of their respective tissue of origin.

In such comparisons it is the differentiation markers *COL1A1*, *RUNX2*, *ALP*, *BSP* and *OC* which are usually taken as key indicators of differences between tissues. However, here we saw minimal difference in the expression of these genes between unfused and fused suture tissues (Fig. 3A). This may reflect the active remodelling nature of the unfused suture tissues which include the actively differentiating osteogenic fronts, compared to the terminally differentiated and less actively remodelling fused suture tissues. Furthermore, in all cases (except *COL1A1*), the fold change induced by either osteogenic medium was in a direction opposite to that observed in vivo.

With respect to the genes which we have identified as being highly differentially expressed during premature suture fusion (Fig. 3B), again neither osteogenic supplement induced in vivo-like differential expression patterns. Only *RBP4* and *WIFI* had fold changes in the same direction, however, *RBP4* was not induced to the same extent and *WIFI* showed more than 100-fold greater fold change particularly under OM + D compared to in vivo fold changes levels.

Discussion

Media supplemented with ascorbic acid and β -glycerophosphate with, and without, dexamethasone are commonly used to induce osteoblast differentiation and mineralised bone nodule formation for cells cultured from rodent and human calvarial tissue (Nefussi et al., 1985; Bellows et al., 1987; Owen et al., 1990; de Pollack et al., 1997; Debais et al., 1998; Malaval et al., 1999; Igarashi et al., 2004; Ratisoontorn et al., 2005). While this in vitro environment produces a bone-like ECM matrix, in vitro mineralisation studies continue to be undertaken using conditions that are dramatically different from those in vivo. Interaction with other tissue types, such as the dura mater, regulates suture cell fate (Opperman, 2000; Spector et al., 2002), while gradients of factors such as *FGF2* within the suture complex regulate the proliferation and differentiation of osteoblastic cells (Iseki et al., 1999). It has also been shown that osteoprogenitors can achieve the same end-point of differentiation via different developmental routes (Madras et al., 2002), suggesting that an in vitro development path could be very different to that which occurs in vivo. Finally, there appear to exist at least two distinct populations of osteoprogenitor cells which, when explanted, respond differently to osteogenic supplements, the more primitive of these only forming a mineralised matrix with the addition of specific inductive stimuli, such as dexamethasone (Turksen and Aubin, 1991). This poses the question whether osteoprogenitor differentiation induced by osteogenic media with and without dexamethasone occurs via the same pathway or whether the different supplements produce the same endpoint via the activation of different genes/pathways?

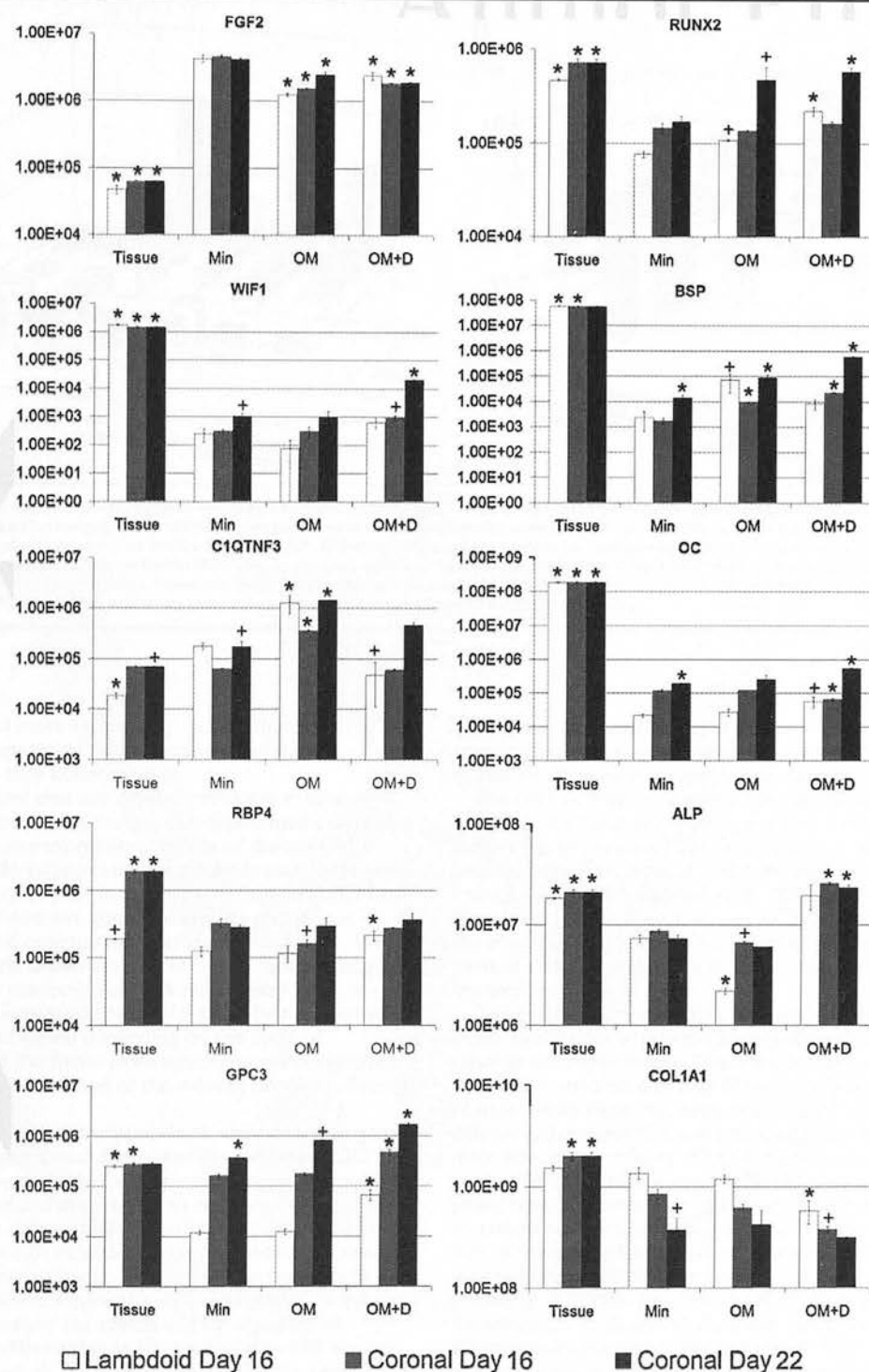


Fig. 2. Expression profiles for 10 genes analysed in cells grown from fused suture tissue from Patient AP under minimal medium, standard osteogenic medium, and standard differentiation medium with 100 nM dexamethasone. Compared to in vivo expression (tissue) all genes were generally down-regulated in cells cultured in minimal medium. The exceptions were *FGF2* and *C1QTNF3* which were up-regulated or unchanged. Neither osteogenic media were able to induce expression to levels seen in tissues. Scale is log₁₀ amplicon copy number per ng cDNA. Significant differential expression (* $P < 0.05$ Bonferroni corrected; + $P < 0.05$ uncorrected) is compared to minimal medium at each time point and for minimal medium at day 22 compared to that at day 16. See Materials and Methods Section for corrected P -values. Absolute expression values represent molecules per ng cDNA.

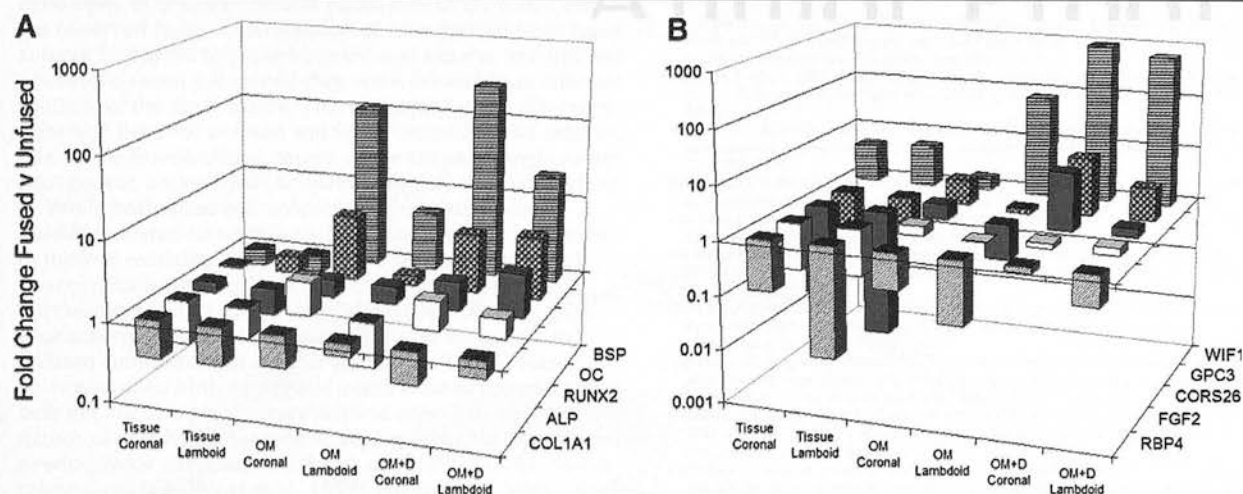


Fig. 3. Differential expression of 10 genes between fused and unfused sutures tissue samples from coronal and lambdoid sutures and expression in the corresponding cell cultures grown in either osteogenic medium or osteogenic medium with dexamethasone. Unfused suture samples are from patient NS and fused suture samples are from patient AP. Differentiation marker genes (A) and genes which are known to be highly differentially expressed during premature suture fusion (B) show, in general, a similar decreased expression in both coronal and lambdoid fused sutures tissues compared to unfused sutures tissues. However, both osteogenic media only recapitulated the direction of differential expression, as observed in vivo for *COL1A1*, *OC* and *RBP4*, and even then this was not to a level which reflected that in vivo.

Furthermore, and most importantly, does either media stimulate gene expression and subsequent cellular differentiation as they occur in vivo?

We hypothesised that any differences in the nature of differentiation produced by various osteogenic media would be reflected in the gene expression profiles of the cells. We confirmed the differentiation of cells grown in each osteogenic medium by staining for the formation of a collagenous ECM and mineralised bone nodules, generally used as phenotypic markers of mature osteoblasts (Ecarot-Charrier et al., 1983; Bellows et al., 1986; Owen et al., 1990). While both osteogenic media stimulated the formation of a mineralised ECM, in general, the gene expression profiles induced by the two media were different and varied depending on the stage of morphogenesis of the tissue from which the cells originated (Table 2). Furthermore, none of the induced profiles reflected those seen in vivo.

Amongst the 10 genes analysed there were only two genes which both osteogenic media consistently modulated: *FGF2* and *CIQTNF3*. *FGF2* was dramatically up-regulated in minimal medium compared to that in vivo, with no change in expression over time. Under differentiating conditions, *FGF2* expression was reduced, although neither osteogenic medium succeeded in reducing it to in vivo levels. This observation is of particular importance as *FGF2* is a common supplement used in in vitro experiments to analyse the effects of FGF signalling on proliferation and differentiation (Debiais et al., 1998; Kalajzic et al., 2003; Choi et al., 2005; Fakhry et al., 2005). Under differentiating conditions, the addition of *FGF2* to culture medium would therefore negate the suppression of *FGF2* by osteogenic supplements and potentially result in *FGF2* levels equal to or greater than observed for minimal media which are already well in excess of that observed in vivo.

The other consistent pattern of gene modulation observed for both unfused and fused suture-derived cells was an increase in *CIQTNF3* expression by standard osteogenic medium and, in general, a decrease in expression compared to minimal medium with the addition of dexamethasone. The inhibition of *CIQTNF3*

expression by dexamethasone favours the hypothesis that these two osteogenic supplements promote differentiation via activating different gene expression cascades.

Differences in gene expression induced by osteogenic media supplemented with dexamethasone and other osteogenic factors has been carried out for a variety of human and rodent primary cells (Bellows et al., 1987; Turksen and Aubin, 1991; de Pollack et al., 1997; Ogston et al., 2002; Igarashi et al., 2004; Zhou et al., 2006). However, our study is the first to compare the effects of media supplementation on human calvarial suture derived cells in comparison to the in vivo expression seen for the explant tissue of origin.

The cellular heterogeneity of calvarial tissues is an important factor to consider when interpreting observations from in vitro calvarial cell experiments. There is increasing evidence from in vivo and in vitro studies that different sub-populations of osteoblasts exist that have unique proliferative and differentiative capacities and that these may be reflected by their gene expression profiles (Turksen and Aubin, 1991; Liu et al., 1997; Candelieri et al., 2001; Madras et al., 2002). The phenotypic and molecular effects of craniosynostosis-causing mutations are often studied by comparing cultured cells derived from prematurely fused calvarial tissue and unaffected calvarial tissues (unfused sutures or bone) from control patients (Lomri et al., 1998; Yousfi et al., 2002; Guenou et al., 2005; Ratisoontorn et al., 2005). As these tissues are comprised of differing proportions of cells at various stages of osteoblastic differentiation, conclusions from such experiments should be viewed with caution. It is extremely unlikely that the relative proportions of these cells and their physical relationship will be maintained in vitro and thus the two tissue-specific cell populations are liable to respond differently to osteogenic media, irrespective of the existence of an underlying mutation.

We analysed the differential effect of supplementation on cells isolated from prematurely fused and unfused calvarial tissues from both the same syndromic craniosynostosis patient and from patients with different aetiologies. Previously, we found no difference in gene expression between suture tissues

at the same stage of morphogenesis from patients with different aetiologies of craniosynostosis (Coussens et al., 2007). Here, we observed faster mineralisation of cells derived from fused sutures compared to those from unfused sutures, and this was consistently seen even when they were derived from different sections of the same suture. This suggests that the differences observed between unfused and fused suture-derived cells are due to the fused/unfused nature of the tissue of origin rather than genetic backgrounds or underlying pathologic mutations.

While both fused and unfused cell populations are de-differentiated to a similar level under short term culture in minimal medium, fused-suture derived cells responded phenotypically more quickly to both osteogenic media. Furthermore, fused suture-derived cells had differing gene expression responses to osteogenic media compared to unfused suture-derived cells. In particular, *RUNX2* was up-regulated by both osteogenic media in fused suture-derived cells but not in unfused suture-derived cells. This unresponsive nature of *RUNX2* to osteogenic supplements has been noted previously for particular subclones of the MC3T3-E1 mouse calvarial cell line (Wang et al., 1999). Additionally, *WIFI*, which is up-regulated during premature suture fusion, is significantly up-regulated by osteogenic media in cells from fused sutures and down-regulated in those from unfused sutures (Table 2). Consistent with these observations is the recent suggestion that *WIFI* has an essential role in initiating terminal osteoblast differentiation (Vaes et al., 2005).

We suggest that the increased responsiveness to osteogenic media of fused suture-derived cells is due to the fact that the majority of the isolated cells were fully differentiated in vivo before being caused to de-differentiate in vitro. Consequently, these cells retain a genetic 'memory'; that is, genes expressed in vivo retain epigenetic markers of expression such as hypomethylation or a characteristic chromatin structure allowing them to respond more quickly to subsequent activation (for review, see Levenson and Sweatt, 2006; Wu and Sun, 2006). Conversely, cells isolated from unfused sutures may represent a population of cells at various stages of the osteoblast lineage, including a significantly large number of progenitor cells that have yet to be stimulated to differentiate by the in vivo environment. Consequently, it takes longer for unfused suture-derived cells to respond to osteogenic supplements but given sufficient time in culture (e.g. 22 days) there is little phenotypic difference between cells isolated from unfused and prematurely fused sutures (Fig. 1). Although comparison of cells derived from different calvarial tissue sources is useful for studying molecular mechanisms in craniosynostosis, our data highlight the need for caution in interpreting in vitro observations and in reliance on an in vitro mineralisation phenotype as an indicator of an in vivo-like osteoblastic phenotype.

In conclusion, our results highlight the variability that exists in in vitro osteoblast differentiation experiments and the limitation of relating in vitro observations to the in vivo situation; that the molecular pathway of osteoblastic differentiation stimulated by dexamethasone is distinct from that stimulated by osteogenic media without dexamethasone; that the same osteogenic medium induces different gene expression in cells isolated from calvarial sutures at different stages of morphogenesis; and that the induced profile and level of gene expression does not directly relate to that seen in vivo. Additional media supplements need to be explored to identify those that can adjust the gene expression profiles of cultured cells to that which occurs in comparable in vivo situations.

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SUMMARY

The first five chapters in this thesis contain information which improves the knowledge of clinical features of craniofacial anomalies and so aids diagnosis. It describes management strategies to improve and enhance our current clinical practice. Finally, it has some long-term outcome measures which can be used to re-define and refine our treatment protocols for each craniofacial condition.

The final chapter reviews attempts using rigorous scientific methods to improve the understanding of disease processes. This is critically important that these studies continue as it is likely that any major changes in clinical practice, as opposed to minor technical modifications, will be achieved by better understanding of disease processes and resultant morphological changes, but particularly the molecular and genetic regulators of intracellular metabolic pathways.

CHAPTER ONE: CRANIOSYNOSTOSIS

A New syndrome with Craniosynostosis and Cleft Palate

Author contributions:

Study design, Collection of data, Manuscript written, Submission,
Revision

The spectrum of Antley-Bixler Syndrome

Author contributions:

Study design, Collection of data, Revision

Anomalous venous drainage in a Case of non-syndromic
craniosynostosis

Author contributions:

Study design, Manuscript written, Submission, Revision

Ophthalmic Findings in Apert Syndrome Prior to Craniofacial Surgery

Author contributions:

Study design, Manuscript co-written, Revision

Differential effects of FGFR2 mutation in Ophthalmic findings in Apert
syndrome

Author contributions: Study design, Manuscript co-written, Revision

Pyrexia after Transcranial Surgery

Author contributions:

Study design, Interpretation of data, Manuscript co-written, Revision

Pyrexia after Transcranial Surgery for Pfeiffer Syndrome

Author contributions:

Study design, Interpretation of data, Manuscript co-written, Revision

Temperature Course After Transcranial Surgery for Apert Syndrome: A Possible Indicator for Postoperative Complication.

Author contributions:

Study design, Interpretation of data, Manuscript co-written, Revision

Management of Cranial deformity following ventricular shunting

Author contributions:

Study design, Manuscript co-written, Revision

Simultaneous multiple vector distraction using different for
craniosynostosis syndromes

Author contributions:

Study design, Manuscript written, Submission, Revision

Ophthalmic sequelae of Crouzon syndrome

Author contributions: Study design, Manuscript revision

Visual outcomes in Apert syndrome following craniofacial surgery: 29 years experience

Author contributions: Study design, Manuscript co-written, Revision

Intellectual outcomes at skeletal maturity of Crouzon, Pfeiffer and Muencke syndromes

Author contributions:

Study design, Manuscript co-written, Revision

Unicoronal synostosis correction: Long term result at skeletal maturity

Author contributions:

Study design, Manuscript written, Submission, Revision

Breast cancer risk is not increased in individuals with *TWIST* 1 mutation confirmed Saethre-Chotzen syndrome: An Australian multicentre study

Author contributions: Collection of data, Manuscript revision

Study design, Manuscript written, Submission, Revision

CHAPTER TWO: FACIAL CLEFTS

Dental findings in parents of children with Cleft lip and palate

Author contributions: Collection of data, Manuscript co-written, Revision

Study design, Manuscript written, Submission, Revision

Children with Submucous Cleft Palate

Author contributions:

Study design, Collection of data, Manuscript written, Submission,
Revision

Traumatic arteriovenous malformation following maxillary Le fort 1
osteotomy

Author contributions: Study design, Manuscript revision

Spinal anomalies in Goldenhar syndrome

Author contributions:

Study design, Collection of data, Manuscript written, Submission,
Revision

Modified costochondral graft in osteotomy in Hemifacial microsomia

Author contributions:

Study design, Manuscript written, Submission, Revision

Van der Woude syndrome: dentofacial features and implications for
clinical practice

Author contributions:

Study design, Interpretation of data, Manuscript co-written, Revision

Author contributions: Study design, Revision

Multidisciplinary management of Opitz G BBB Syndrome

Author contributions:

Study design, Collection of data, Manuscript co-written, Revision

From birth to maturity: A group of patients who have completed their protocol management. Part II Isolated Cleft Palate.

Author contributions:

Study design, Interpretation of data, Manuscript written, Submission, Revision

From birth to maturity: A group of patients who have completed their protocol management. Part III Bilateral Cleft Lip-Palate.

Author contributions:

Study design, Interpretation of data, Manuscript co-written, Revision

Mandibular lengthening by distraction for Treacher-Collins syndrome:

The long term result

Author contributions:

Study design, Collection of data, Manuscript written, Submission, Revision

Treacher-Collins syndrome: Protocol management from birth to maturity

Author contributions: Study design, Revision

Longitudinal outcome of Pharyngoplasty

Author contributions:

Study design, Collection of data, Manuscript written, Submission,
Revision

CHAPTER THREE: TRAUMA

Fractures of the facial skeleton in Children

Author contributions:

Study design, Collection of data, Manuscript written, Submission,
Revision

Orbital floor fractures in young children

Author contributions:

Study design, Collection of data, Manuscript written, Submission,
Revision

Hyperostosis as a late sequel of parasymphyseal mandibular fractures in
children

Author contributions:

Study design, Collection of data, Manuscript written, Submission,
Revision

Facial fractures resulting from dog bites

Author contributions:

Study design, Manuscript written, Submission, Revision

A pitfall in the radiological diagnosis of paediatric mandibular condylar fractures

Author contributions:

Study design, Collection of data, Manuscript co-written, Revision

Medial Orbital wall fractures in children

Author contributions:

Study design, Collection of data, Manuscript written, Submission, Revision

CHAPTER FOUR: TUMOURS

Congenital gingival granular cell tumour

Author contributions:

Study design, Collection of data, Manuscript written, Submission, Revision

Odontogenic keratocyst in a five year old child – a rare cause of a maxillary swelling in the paediatric population

Author contributions: Study design, Revision

Familial nasal dermoid cysts

Author contributions:

Study design, Collection of data, Manuscript written, Submission,
Revision

Teratomas of the head and neck region

Author contributions:

Study design, Collection of data, Manuscript written, Submission,
Revision

Sentinal node biopsy in the clinical management of
haemangioendothelioma

Author contributions:

Study design, Collection of data, Manuscript written, Submission,
Revision

Modified transoral approach for resection of skull base cordomas in
children

Author contributions:

Study design, Collection of data, Manuscript revision

Rhabdomyosarcoma of the mandible – long term management from
childhood to adulthood

Author contributions: Study design, Collection of data, Revision

CHAPTER FIVE : LOCOMOTOR

Familial Parry-Romberg disease

Author contributions:

Study design, Collection of data, Manuscript written, Submission,
Revision

The Spectrum of anomalies in pterygium syndrome

Author contributions:

Study design, Collection of data, Manuscript co-written, Revision

The management of facial dysmorphism associated with Nemaline
myopathy

Author contributions:

Study design, Collection of data, Manuscript written, Submission,
Revision

An unusual complication of mandibular distraction

Author contributions:

Study design, Manuscript co-written, Revision

Craniofacial fibrous dysplasia: clinical characteristics and long term
outcomes

Author contributions:

Study design, Collection of data, Manuscript revision

CHAPTER SIX: BASIC SCIENCE

3D CT Cephalometry of plagiocephaly: Asymmetry and shape analysis

Author contributions:

Collection and Interpretation of data, Manuscript revision

A 3D CT analysis of the cervical spine in patients with cleft lip and palate

Author contributions: Interpretation of data, Manuscript revision

A 3D CT analysis of the hyoid bone in patients with cleft lip and palate

Author contributions: Interpretation of data, Manuscript revision

A 3D CT analysis of craniofacial asymmetry in Malaysian infants with cleft lip and palate

Author contributions: Manuscript revision

Intracranial Volume of patients with non-syndromic craniosynostosis

Author contributions:

Study design, Interpretation of data, Manuscript co-written, Revision

Dental anomalies in Apert syndrome: A histological study

Author contributions:

Study design, Manuscript co-written, Revision

Identification of genes differentially expressed by premature fused human sutures using a novel in vivo-invitro approach

Author contributions: Study design, Primary cell culture, Revision

Unravelling the molecular control of calvarial suture fusion in children with craniosynostosis

Author contributions: Study design, Primary cell culture, Revision

In vitro differentiation of human calvarial suture derived cells with and without dexamethasone does not influence in vivo-like expression

Author contributions: Study design, Primary cell culture, Revision